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SEPARATION OF STARCH AND GLUTEN

VI. STARCH RECOVERY AND PURIFICATION[1]

BY G. A. ADAMS[2]

Abstract

Separation and recovery of starch and gluten from wheat flour was investigated on 98-lb. lots of flour. In most experiments, starch was recovered from the starch slurry (sp. gr. 6°Bé) by tabling. Most efficient starch recovery was obtained with a feed rate of 10 lb. of slurry per foot width of table per min., a slurry density of 6°Bé and a pH of 6.0. Under the best conditions, about 55-60% of the starch in the slurry was recovered on the table; retabling of the "tailings" increased the yield to 65-70%. The recovered starch was of prime quality and had a protein content of 0.5%. Starch recovery by centrifuging slurries was almost quantitative but the starch contained 0.75-1.0% protein. Rewashing of the crude centrifuged starch with water, or preferably with water containing sulphur dioxide, reduced the protein content considerably. Eighty per cent of the starch in the starch table 'tailings' was recovered by centrifuging but the product was inferior owing to high 'squeegee' starch and protein content. Normal recovery of prime quality starch was made from spontaneously fermented starch slurries (pH 3.6). Crude gluten containing about 66% moisture was recovered in almost quantitative yield. After drying on a double drum dryer the final product contained about 12% starch and 80.0% protein. The overflow liquors from the starch centrifuge were successfully recycled through the process 10 times as make-up water for the dough preparation and slurrying operations. If sulphur dioxide is present in these liquors their reuse is precluded because of the solubilizing effect on gluten.

Introduction

Wheat starch has been an article of commerce since ancient times, although it has gradually lost its importance as a major source of the world's starch supply. This diminution in importance is attributable to two main causes: starch can be produced more cheaply from other sources; and technical difficulties in effectively separating starch from the tenacious gluten with which it is associated. In Canada, wheat is the most abundant starch bearing crop—often available in surplus quantities—yet a wheat starch industry of major proportion has not developed owing to a lack of adequate technical methods to make wheat starch competitive with other starches. Inadequate utilization of the by-products, principally gluten, has also contributed to the relatively

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as Paper No. 109 on the Industrial Utilization of Wastes and Surpluses and as N.R.C. No. 2379.
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higher cost of wheat starch. The shortage of corn starch during World War II stimulated an interest in the production of wheat starch and considerable progress was achieved in the development of more efficient methods of production.

During the past 10 years several improved methods of separation have been developed and these have been recently reviewed by Kerr (5, p. 102). Of these, two processes that are similar in principle appear to offer good commercial possibilities. The 'screening' process developed by Shewfelt and Adams (7) and the 'batter' process developed by Hilbert *et al.* (4) both involve preparation of a soft dough or batter from flour. On slurrying with additional water, followed by screening, the dough is readily separated into crude gluten and starch milk. Further processing of both fractions produces final products of high quality in satisfactory yield.

The simplicity and efficacy of the screening process in the laboratory prompted further investigation of its feasibility on a larger scale and, in particular, evaluation of suitable starch recovery procedures. The tabling method for starch recovery was selected as it is widely used in commercial practice. Recovery by centrifuging was also studied, although restricted to laboratory scale equipment. Minor subjects of investigation were purification of starch by treatment with sulphur dioxide, recovery of starch from table 'tailings', purification of crude gluten and reuse of starch liquors in the process.

Materials and Methods

Starch–Gluten Separation Equipment

The layout of the equipment is shown in Fig. 1. The dough mixer was a vertical tank 27 in. diameter, 46 in. high (capacity 95 gal.) equipped with a heavy duty agitator driven at 54 r.p.m. by a 2 h.p. motor. The bottom of the tank was closed by a 6 in. gate valve, which discharged into a hemicylindrical holding tank 36 in. in diameter × 72 in. long (capacity 250 gal.). The contents of the holding tank were transferred by a Jabsco rubber impeller pump through a 1 in. line to a Rotex screener fitted with a 150 mesh stainless steel screen. The starch slurry was drained off from the screener to a 550 gal. storage tank and the gluten was discharged into a 9 gal. washing tank equipped with a high speed agitator (1/4 h.p. 1725 r.p.m., two 4 in. propellers). The overflow from the tank was pumped to a 45 gal. rewashing tank similarly equipped with an agitator. From this vessel the slurried gluten was recycled to the Rotex screener.

Starch Recovery Equipment

The starch recovery equipment consisted primarily of a starch table with a constant feed device and the necessary storage tanks. The starch storage tank was connected through a centrifugal pump and pipe line to a second, smaller storage tank that supplied a constant level head box. The head box was equipped with an outlet line fitted with interchangeable orifices (1/4, 3/16,

and 1/8 in. diameter) for regulating the rate of flow to the starch table. The starch table, of wooden construction (cypress), was 85 ft. long, 1 ft. wide, and 4 in. deep and had a slope of 2 in. over the entire length. The tailings overflow was pumped to a holding tank from which it could be recycled to the table.

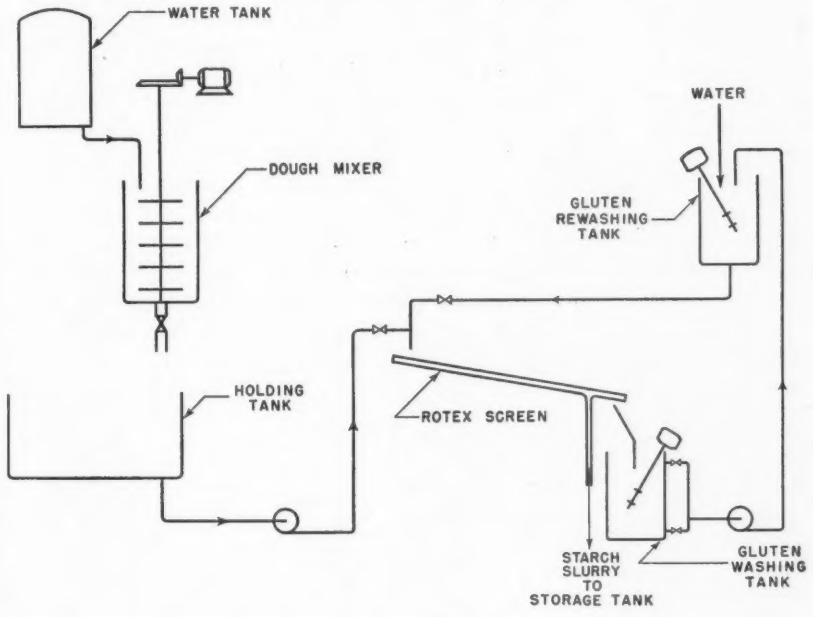

FIG. 1. *Diagram of starch–gluten separation process.*

The wet table starch was dried on metal trays in a drying chamber heated with circulating heated air. The dried product was powdered by passage through a hammer mill. For recovery of starch by centrifuge, the equipment consisted of a 12 in. solid bowl mounted on a large laboratory centrifuge.

Methods of Analysis

The yield of product and analytical data are reported on a dry basis unless otherwise stated. Moisture in starch was determined by drying the samples in a vacuum oven at 105°C. for 16 hr. Gluten samples, which had been previously drum dried, were dried to constant weight in a vacuum oven at 105°C. The Kjeldahl method was used for determining nitrogen and the results multiplied by 5.7 to calculate the wheat protein. Starch was determined in flour, starch, and gluten samples by the methods described by Clendenning (1, 2).

The flour used in the experiments was a commercial patent product containing starch 67.1%, protein 13.0%, gluten 12.4%, and moisture 15.0%.

Experimental Methods and Results

Separation of Starch and Gluten

The separation procedure consisted essentially of the preparation of a thin dough or batter by mixing flour with about 80% of its weight of water. After addition of a larger volume of water to the dough, mechanical agitation of the mixture caused the starch to be washed out of the gluten masses, from which it was removed by screening. Dough mixing and slurrying operations were carried out as batch operations using one bag of flour (98 lb.) as the unit quantity of starting material. Seventy-six pounds of water at 25°C. was placed in the mixing tank and the flour added with agitation and the mixing continued for 20 min. Two hundred and seventy-five pounds of water was then added rapidly with stirring and the stirring continued for a period of three minutes or until the dough mass had broken up into small curds and most of the starch was in suspension in the water. The time of agitation was found to vary with the type of mixer and can be best determined by trial runs. Undermixing gave inadequate separation of starch from gluten, while overmixing tended to disperse the gluten too finely for proper screening.

After the gluten was dispersed, the whole mix was immediately dumped into the holding tank. Gentle agitation of the mixture in the tank prevented settling out of the gluten; agitation ahead of the pump is important, otherwise clogging may occur. The mixture was then pumped to the Rotex screener where the separation into crude gluten and starch milk was made. The gluten came over the end of the screen into the gluten washing tank containing 8 gal. of water. The agitator broke up the gluten mass again and effected further removal of starch. The gluten slurry in excess of 8 gal. was constantly taken off through an overflow line and pumped to the gluten rewashing tank, containing 15 gal. of water, where further washing took place. After all the original slurry had been screened, the remainder of the gluten slurry in the washing tank was pumped into the rewashing tank and agitated for five minutes. The washed gluten was then passed over the Rotex screener and recovered as a cohesive mass from the screen. The gluten mass was placed on a sloping drainboard and the excess of free water drained off. Manual kneading assisted in the removal of water. A representative portion of the gluten from each experiment was dried on a double drum dryer at 70 p.s.i. (steam pressure) and reserved for analysis for moisture, protein, and starch.

Composition of Starch Milk and Gluten from Flour

Study of the various experimental factors affecting the recovery of starch and gluten required knowledge of the starch and gluten content of the various fractions obtained from the primary separation. Typical results from such a separation are given in Table I.

TABLE I

YIELD AND COMPOSITION OF STARCH MILK AND CRUDE GLUTEN FROM ONE BAG OF FLOUR (98 LB.)

Item	Weight, lb.	Composition, %
Weight of dough slurry	449	
Weight of starch slurry	535	
Solids in starch slurry	69.55	13.0
Starch in solids	63.99	92.0
Protein in solids	2.00	2.88
Weight of crude wet gluten	40	
Weight of crude dry gluten	13.2	33
Starch in crude dry gluten	1.65	12.5
Protein in crude dry gluten	10.56	80.0
Fat (alcohol soluble) in crude dry gluten	0.92	7.0
Starch in flour	65.76	67.1
Starch accounted for	65.63	99.7

FACTORS AFFECTING STARCH RECOVERY

Effect of Rate of Feed to Table on Starch Recovery

Starch slurry from the separation process was run to the starch table at a constant rate of flow through the various sized orifices. Starch slurry of 6.0° Bé sp. gr. was delivered at rates of 9.1, 6.4, and 3.0 lb. per min. respectively. After all the starch slurry had been run on to the table, a short draining period permitted further settling of the starch. Several methods of washing the starch were tried and the most effective was found to be a light spray of water over the surface of the starch, directed in such a way as to sweep away the gluten and other impurities that had accumulated on the starch. The wet starch (45% moisture), after a further draining period, was removed, dried, and analyzed. The tailings from the first starch recovery were retabled by the same method as used for the original slurry. The results of the complete experiment are given in Table II. They show, in general, that a slow rate of feed to the

TABLE II

EFFECT OF RATE OF FEED OF SLURRIES TO STARCH TABLE ON RECOVERY AND PURITY OF STARCH

(Sp. gr. of slurry 6°Bé)

	First table starch*			Second table starch**			
Rate of feed, lb./min.	Recovery of pure starch, %	Protein, %	Starch purity, %	Recovery of pure starch, % of original feed	Protein, %	Starch purity, %	Total pure starch recovered, % of original slurry
9.1	56.2	0.45	96.65	10.7	0.49	95.38	66.9
6.4	65.0	0.36	96.50	5.7	0.42	97.00	70.7
3.0	68.7	0.45	97.90	3.6	0.87	95.30	72.3

* *Starch recovered on table from original slurry.*
** *Starch recovered on table from retabling of tailings.*

table resulted in an increased recovery of starch. However, retabling of the tailings obtained in the first pass resulted in only a small additional recovery of starch. Initial starch recovery tended to vary inversely with rate of feed; the effect was reversed for the tailing starch recovery. The total yield of starch recovered from a feed rate of 3 lb. per min. was only 6% greater than from a feed rate of 9 lb. per min. This apparent advantage of the slow rate of feed was offset by a greatly increased overall processing time.

The effect of the different rates of tabling on the purity of the starch was small. No marked differences in the protein content of the first table starches were found; the purity of the starch was satisfactory in all experiments. The starch recovered from the tailings was of a purity similar to that of the first starch with the exception of the tailings recovered from the slowest rate of feed, which was ineffective in floating away the particles of gluten and insoluble pentosan material. There was no apparent advantage in using a slow rate of feed in tabling the starch slurry; a fast rate of flow combined with retabling of the tailings is preferable.

Effect of Slurry Densities on Starch Recovery

Starch slurries having specific gravities of 3°, 6°, 9°, and 12°Bé respectively were studied. The slurry as yielded by the separation process was 6°Bé. A slurry of 3°Bé was prepared by suitable dilution of the 6° slurry and the more concentrated slurries were prepared by withdrawing the appropriate amount of liquor from the 6° slurry after a short settling period. All slurries were tabled at approximately the same rate (9 lb. per min.). The results are given in Table III. In general, they show that the highest yields of starch were obtained

TABLE III

EFFECT OF SPECIFIC GRAVITY OF SLURRIES ON RECOVERY AND PURITY OF STARCH

Specific Gravity, °Bé	Recovery of pure starch, %	Starch purity, %	Protein, %
3	66.8	98.35	0.45
6	56.2	96.65	0.45
9	38.5	96.25	0.48
12	20.3	96.25	0.50

from the low gravity slurries. No retabling of tailings was made. The yield of starch obtained from the 3°Bé slurry was 66.8% which is about equal to that obtained from two tabling operations in the previous experiment (Table II). It appears likely that this figure represents approximately the maximum amount of starch that can be readily recovered by the tabling method used. While the low gravity slurry has a slight advantage in starch yield, it does not compensate for the large increase in volume that must be handled. The high gravity slurries (9° and 12°Bé) gave low yields and showed the impracticability of concentrating wheat starch slurries prior to tabling. Since there was little

difference in the purity of the starch recovered in all experiments it appeared that a slurry of 6°Bé has the best consistency for practical recovery.

Effect of pH of Starch Slurry on Starch Recovery

The pH of the slurry as received from the separation process was normally 6.0. The pH was adjusted downwards to 3.2 with hydrochloric acid and upwards with sodium hydroxide to 8.7. The slurries were tabled at the rate of 9 lb. per min., and the starch washed, recovered, and dried by the prescribed methods. No marked differences in behavior of the starch slurries was observed except that the alkaline slurries settled more slowly than the acid suspensions. The data on yield and purity are given in Table IV. Only minor differences in

TABLE IV

EFFECT OF pH OF SLURRIES ON RECOVERY AND PURITY OF STARCH

pH	Recovery of pure starch	Starch purity, %	Protein, %
3.20	53.8	98.70	0.39
4.05	54.8	98.40	0.41
5.10	56.8	97.60	0.37
6.10	56.2	97.80	0.45
7.35	54.7	98.40	0.37
8.70	55.3	98.70	0.37

yield occurred at the various pH levels; there was a slight tendency for the optimum yield to occur in the range pH 5.0–6.0. Starch purity was lowest in the pH range of maximum recovery. Apparently solubilization of impurities (mostly protein) occurred at a lower and higher pH values. The extreme ranges of pH had no observable deleterious effects on starch quality. It was concluded that the pH of 6.0 obtained in normal separation is the most advantageous for starch recovery.

Purity of Starch at Intervals Along the Table

It was observed that over 80% of the collected starch settled out in the first 60 ft. of the table when the starch slurry was run to the table at the rate of 9 lb. per minute. Since the starch that was first deposited appeared whiter and freer of gluten and 'squeegee' material, it was decided to analyze the starch at 10-ft. intervals along the length of the table to determine its comparative purity. The results are given in Table V. The upper end of the table contained the purest starch, the nitrogen content being lowest in the first 30 ft. There was a gradual decrease in purity of the starch over the length of the table owing to redeposition of impurities. In commercial practice, in which heavier loadings of the table would be used, it might be advantageous to wash the impure starch from the lower end of the table and recycle it with the tailings.

TABLE V

PURITY OF STARCH ALONG SETTLING TABLE

Distance from head of table, ft.	Protein in starch, %	Purity of starch, %
0 (feed slurry)	2.46	86.02
10	0.36	94.52
20	0.37	94.42
30	0.37	94.35
40	0.40	94.10
50	0.41	93.95
60	0.49	93.60
70	0.43	93.75
80	0.53	93.42

Retabling of the First Table Starch

As a purification measure, the first tabled starch from a typical experiment was completely removed from the table, made up with water to its original specific gravity and retabled. The results showed a reduction in protein content of starch from an original value of 0.53 to 0.15% and an increase in starch purity from 94.2% to 98.0%. However, the starch did not settle as readily on retabling and about 10% was carried off in the tailings.

Recovery of Starch by Centrifuging

Although the main purpose of the present study was to determine the efficiency of starch recovery by tabling, a few experiments were carried out in which the starch was recovered by centrifuge. Since only laboratory equipment was available, the experiments gave only a limited demonstration of the results to be expected on commercial equipment. The starch slurry (6°Bé) was fed by gravity through a drawn glass tip to the spreading head of the centrifuge bowl. The rate of feed varied; as the bowl became filled with solids the rate of feed was decreased. About 25 liters of slurry were centrifuged per hr. When the bowl was almost filled with solids and the effluent began to show traces of starch, the operation was stopped, the starch removed and dried. A cross section of the starch layer in the bowl showed the great bulk of the material to be clean white starch with an inner layer of impure starch containing bran and gluten particles along with a soft layer of 'squeegee' starch. In a typical experiment the recovery of solids from the starch slurry was 98%; the starch was 96.3% pure and contained 0.87% protein. The higher protein content of the centrifuged starch as compared with tabled starch was due to embedding of gluten particles in centrifuged starch, which in tabled starch were floated off. Rewashing and recentrifuging of the centrifuged starch were investigated as a means of reducing the protein content. Four kilograms of wet centrifuged starch was slurried with 15 kilo. of tap water for 10 min. and again recovered by centrifuging. The results were as follows:

	Solids recovery, %	Purity, %	Protein in starch, %
Original starch	98.0	96.3	0.87
Washed starch	91.9	96.9	0.71

The rewashing procedure resulted in a considerable loss of starch but this was due to mechanical handling and would be minimized in large-scale operations. Only a slight improvement in starch purity was achieved although a considerable reduction in protein was realized. In other experiments, a reduction to less than 0.5% protein in starch was effected by washing the starch cake in the centrifuge bowl with water. The impurities being in the inner layer were swept away by the water but only at the expense of losing some starch. In a commercial operation, a compromise would have to be reached between maximum recovery of starch solids and purity.

In the centrifuge experiments, it was found that slurries of greater gravity than 6°Bé gave increasingly impure starch. In general, the more dilute the slurry the higher the purity of the centrifuged starch. If the inner 'squeegee' layer of starch is removed from the centrifuged product, the remainder, after suspension in water, can be readily filtered. Filtration of the original slurry is not possible, owing to presence of the 'squeegee' fraction.

Purification of Centrifuged Starch with Sulphur Dioxide

Purification of the centrifuged starch was attempted by reslurrying with water containing sulphur dioxide. The results were as follows:

	pH	Recovery, %	Purity, %	Protein in starch, %
Original starch	6.05	94.4	94.6	0.88
SO_2 treated starch	5.00	94.2	94.8	0.84
SO_2 treated starch	3.88	94.8	95.5	0.61

While no marked differences in recovery and starch purity resulted from the sulphur dioxide treatment, there was a significant reduction in protein content of the starch at pH 3.88. Although sulphur dioxide has the twofold advantage of solubilizing gluten and retarding microbial growth, it has the decided disadvantage of interfering with the separation process if the supernatant liquors are recycled to the dough making operation.

Centrifuging of Starch Table 'Tailings'

The starch tailings from the starch table contained approximately 30% of the starch in the original slurry. Owing to the presence of a large proportion of the 'squeegee' material this starch was difficult to recover by retabling and recovery by centrifugation was investigated. An aliquot of the first tailings was

centrifuged in the laboratory-size imperforate bowl. The results showed that 80% of the starch in the tailings was recovered. However, this product contained 2.0% protein and was of inferior quality. In a typical experiment the combination of tabling with centrifugation of the tailings afforded a total recovery of 93% of the available starch.

Recovery of Starch from Fermented Slurries

Since spontaneous fermentation occurs in starch slurries on standing, especially in warm weather, the effect of fermentation on starch recovery and purity was investigated. A starch slurry separated in the usual way was held overnight at 78°F. The pH decreased from 6.1 to 3.6 during a 16 hr. period. The slurry (sp. gr. 6°Bé) was then tabled as before at the rate of 9 lb. per min. A starch yield of 53.8% was obtained on one tabling; this result compares favorably with those obtained using fresh starch slurries. The starch solids contained 98.7% starch and 0.47% protein. Apparently, natural fermentation of a relatively short duration did not cause any diminution in either starch yield or purity.

Purification of Crude Gluten

Crude gluten as recovered from the Rotex screener in the primary separation step contained approximately 25% starch. The arbitrary procedure adopted in the first pilot plant run, to reduce this starch content, was to agitate the crude gluten (35 lb. net weight) in 15 gal. of water for 20 min. This treatment reduced the starch content to approximately 15%. To examine the effect of gluten washing more exactly, a study of a prolonged washing period was made, with starch analyses being carried out at regular intervals. Thirty-five pounds of crude wet gluten (yield from 98 lb. of flour), as recovered from the initial separation, was vigorously agitated in 30 gal. of water with a high speed stirrer. A sample of gluten was removed before agitation began and at the end of each 10 min. period thereafter for 50 min. After it was rinsed with fresh water the gluten was dried and analyzed for starch content. The results, given in Table VI, show that a 10 min. washing period reduced the starch content by approximately one-half; additional washing up to a 50 min. period caused no further significant decrease in the starch content.

TABLE VI

Effect of washing time on starch removal from crude gluten

Washing time, min.	Starch in gluten, %
0	24.65
10	13.87
20	12.48
30	12.00
40	11.97
50	11.75

In a second experiment, the effect of washing the crude gluten in successive changes of water was investigated. The crude wet gluten (35 lb.) was agitated with a high speed stirrer for 10 min. in 6 gal. of water. The mixture was then passed over the Rotex screener and the gluten recovered, rinsed with water, and a sample taken for starch analysis. In all, the gluten was subjected to five successive washing treatments. The results of the starch analysis are given in Table VII. They show that gluten can be washed substantially free of starch

TABLE VII

EFFECT OF SUCCESSIVE WASHES IN WATER ON STARCH REMOVAL FROM CRUDE GLUTEN

Number of washings	Starch in gluten, %
0	26.98
1	6.20
2	4.63
3	2.49
4	0.62
5	0.31

by vigorous agitation in successive batches of fresh water. However, with the equipment used, this operation was difficult because after removal of starch the gluten became very tough and tenacious and tended to wind up on the stirrer shaft. The greater effectiveness of the first 10 min. washing period over that in the previous experiment (Table VI) was due in part to separation of the gluten into small pieces by hand. While washing in fresh batches of water produced a relatively pure gluten, the number of extra operations involved in addition to the washing difficulties required a compromise treatment. This was achieved by running a small stream of water into the washing tank that received the gluten from the screener. The excess was pumped off and, at the end of the screening operation, the entire gluten suspension was pumped into the rewashing tank and agitated for a further 15 min. This treatment yielded glutens containing approximately 12% starch.

The starch slurries recovered from the gluten washing operations contained about 11.9% protein in the dry solids as compared with 2.35% in the dry solids in the original starch slurries. In commercial operation, it would be preferable to keep the gluten washings separate from the main bulk of the starch slurries in order to prepare a higher quality starch.

Drying Gluten

Gluten was recovered for analytical purposes by drying on a laboratory double drum dryer to a moisture content of 7-10%. The same equipment of commercial size may be used for preparing denatured gluten on an industrial scale. As recently reported (6), spray drying can yield a gluten with good baking quality. Current investigations in this laboratory have shown that a partially denatured product can be obtained by flash drying.

Reuse of Supernatant Liquors in the Separation Process

Efficient commercial operation of the starch gluten separation and recovery process involves reuse of the liquors from which the starch has been recovered. These should re-enter the process as make-up water for the initial separation stage and, if feasible and economic, should be eventually evaporated for solids recovery. A laboratory investigation on the recycling of the liquors through the process was made. Three kilograms of flour was prepared as a dough, slurried with water, and the usual separation made into starch slurry and crude gluten. The starch was recovered by centrifuging and the supernatant liquor allowed to stand overnight in the cold. An almost clear liquor was siphoned off from a small amount of solids that settled out on standing. The liquor was used in the dough slurrying stage of the next separation experiment. The reuse of the supernatant liquors was repeated nine times in succession, and the effect on starch recovery and purity was determined by analysis. Each batch of centrifuge liquor was analyzed for total solids and protein in the solids. In addition, some samples of the solids that settled out of the liquor on standing were analyzed for protein and starch.

TABLE VIII

REUSE OF CENTRIFUGED STARCH LIQUOR IN THE STARCH–GLUTEN SEPARATION

No. of times liquor recycled	Pure starch recovery, %	Starch purity, %	Protein in starch, %	Decantate from centrifuged liquor	
				Solids, %	Protein in solids, %
None	96.3	96.1	0.82	1.22	19.34
1	89.5	96.2	0.72	1.87	18.40
2	89.8	95.5	0.82	2.31	18.45
3	89.0	94.2	0.87	2.54	18.32
4	88.5	94.8	0.79	2.75	18.00
5	91.6	93.4	0.81	2.90	17.86
6	89.6	93.4	0.86	3.11	17.20
7	88.3	93.7	0.80	3.07	17.54
8	91.4	93.5	0.88	3.01	18.50
9	91.4	93.9	0.81	3.10	19.34

The results (Table VIII) show, in general, that the liquors from the starch recovery are suitable for use in the slurrying step in the starch gluten separation. Some decrease in starch recovery was found as determined by the weight of starch recovered in the centrifuge bowl. The loss was accounted for by the increased starch that settled out from the supernatant liquors on standing. This starch, though relatively high in protein content (2-3%), could be purified by rewashing and centrifuging. The purity of the main starch samples recovered from successive experiments decreased although the protein content remained almost constant. Apparently pentosan material accumulated in the starch. Reworking of the starch with water and centrifuging readily increased the starch purity to about 98%. The supernatant liquors gradually increased in solids content, and, since the solids contained 18% protein, consideration might be given in a commercial process to evaporation of the liquors.

The reuse of centrifuged liquors for a total of nine successive experiments had no harmful effect on the separation of the starch and gluten; however, the use of fermented liquors (pH 3.0-4.0) for dough preparation and slurrying caused solubilization of gluten and the recovered starch was high in protein. If during the processing of the starch liquors, the pH became less than 6.0, adjustment with sodium hydroxide to the original pH permitted satisfactory use of the liquors for the separation procedure.

Use of Sulphur Dioxide in the Process

Although partial neutralization of fermented starch liquors permitted their reuse in the process, it would be desirable to avoid fermentation entirely during the recovery stages. One of the available cheap agents for controlling spontaneous fermentation in the starch industry is sulphur dioxide and its effect on the described process was studied. Various amounts of an aqueous solution of sulphur dioxide were added to the water used for the dough making and slurrying procedures. A normal separation, without sulphur dioxide, served as a control experiment. The results are given in Table IX. In general, they

TABLE IX

EFFECT OF VARIOUS AMOUNTS OF SULPHUR DIOXIDE ON THE SEPARATION OF STARCH AND GLUTEN

Solution for dough making and slurrying		Final slurry		Condition of gluten
	pH	SO_2, %	pH	
Tap water	7.65	.....	5.90	Tough, elastic
Water + SO_2	2.00	0.151	2.46	Solubilized
Water + SO_2	2.23	0.085	2.85	Solubilized
Water + SO_2	2.35	0.038	3.90	Partly solubilized
Water + SO_2	2.62	0.009	4.78	Partly solubilized
Water + SO_2	3.25	0.004	5.22	Partly solubilized
Water + SO_2	4.64	0.002	5.42	Soft, sticky, unscreenable
Water + SO_2	5.70	0.001	5.90	Soft, sticky, normal when rinsed in water

show that even minute quantities of sulphur dioxide in the dough making and slurrying stages caused at least partial solubilization of the gluten. If not solubilized outright, the gluten was rendered soft and sticky and hence unscreenable. The greatest amount of sulphur dioxide that could be tolerated in the process was 0.001%, giving a pH of 5.90. However, this small quantity of sulphur dioxide gave little or no protection against microbial growth.

The feasibility of using neutralized centrifuged liquors that had been treated with sulphur dioxide was also investigated. Liquors containing 0.01% sulphur dioxide, after being brought to neutrality with sodium hydroxide, were found unsatisfactory for gluten separation; the gluten was soft and difficult to screen. Smaller amounts of sulphur dioxide could be tolerated after neutralization but served no useful purpose as fermentation inhibitors. If sulphur dioxide were used in the process for either control of microbial growth or for reducing the

protein in the starch, the effluent liquors could not be reused in the process; however, it would be possible to recover the crude starch by tabling or centrifuging and to treat it with sulphur dioxide in a separate purification step.

Summary and Conclusions

The separation of starch milk and crude gluten from wheat flour has been accomplished on small commercial equipment of simple design without undue difficulty. Continuous operation in a commercial plant would obviously require a number of dough mixers to maintain a constant supply of dough. Consideration might also be given to a type of continuous dough mixer now commercially available; such a unit could be supplemented by a continuous slurrying device to separate the starch from the dough. The screening operation is relatively free from trouble provided the screen is kept clean and is wetted with water before use. General precautions regarding cleanliness must be taken in all parts of the plant to ensure high quality products.

While rewashing of gluten from the screen has been accomplished directly by a high speed agitator in water, insertion of a shredding or extrusion operation before the agitator has also proved effective. The main difficulty to be avoided is winding of the gluten on the shaft of the agitator. The starch recovered from rewashed gluten is abnormally high in protein content and it is recommended that this fraction be recovered separately.

The recovery of starch was made by a tabling operation similar to the commercial procedure; in addition small batch lots were recovered by centrifuging. One tabling operation recovered 55-60% of the starch, of prime quality, and this yield could be increased to 65-70% by retabling of the tailings. Starch slurries having a specific gravity of 6°Bé fed at the rate of 9 lb. per min. per ft. width of table provided the most advantageous conditions for tabling. No advantage in recovery was found by changing the normal pH of the slurry (6.0). Increasing the length of the table over that used (85 ft.) would appear to be of little value in recovery, as the bulk of the starch settled out in about 60 ft. The recovery of a maximum of 70% of the starch on the table left a remainder of 30% in the tailings from which recovery was difficult. The presence of the 'squeegee' fraction in the tailings made settling slow and incomplete. Centrifuging of the tailings recovered about 80% of the starch but the quality was inferior owing to high protein and pentosan content. Although treatment with sulphur dioxide decreased the protein content, it did not remove pentosans at the concentration used. Clendenning and Wright reduced both the protein and pentosan content of wheat starch to low levels by digesting the crude starch slurries in dilute acid (pH 1.8-2.0) for 24 hr. at 40-45°C.(3). This drastic treatment caused no appreciable reduction in starch recovery but did lower paste viscosity; the usefulness of the purified starch for syrup-making purposes was not impaired.

The nature of the 'squeegee' starch makes it a useful source of adhesives and binders. After the removal of as much starch as possible, both by tabling and centrifuging, the remainder could be dried for use by the adhesive trade.

While the first table starch (prime quality) was an excellent material for preparing wheat syrups, the 'squeegee' material was unsatisfactory, producing syrups difficult to clarify and maintain colorless. The entire starch fraction is quite suitable for fermentation processes.

Sulphur dioxide is a useful agent for removing protein from starch in the purification procedure; it is also useful for controlling spontaneous fermentation in the starch slurries. However, the presence of sulphur dioxide in the effluent liquors precludes their reuse in the dough mixing and slurrying operation; the presence of sulphite even in small quantities causes solubilization of the gluten. The table liquors may be satisfactorily recycled to the doughing and slurrying stages, thereby reducing the volume of disposal liquors; if the solids accumulated were judged sufficient, the liquors might be evaporated for protein recovery as feed. Fermentation of the starch liquors does not prevent their reuse; adjustment to pH 6.0 prevents their interference with the dough-mixing operation.

Handling and drying of starch are well developed commercial processes and received no special attention in the study.

Crude gluten was recovered almost quantitatively and in a substantially undenatured state. The subsequent uses for which the gluten is intended determines the method of drying. For all purposes except baking the gluten can be dried at relatively high temperatures on drum or roller type dryers. For baking purposes, denaturation must be avoided and the gluten is dried at relatively low temperatures or at high temperatures for a very short period of time. Vacuum and spray drying (6) are two practical methods of drying gluten with minimum denaturation.

In the present study, the practicability of the process from a technical standpoint was demonstrated. The plant was too small and inefficient to give useful engineering data. Factors that would influence a decision to establish a wheat starch plant such as cost of site, equipment, power, labor, development of markets, etc., are outside the scope of this work.

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PSYCHROPHILIC BACTERIA IN EDMONTON MILK AND CREAM

I. NUMBERS[1]

By I. E. Erdman[2] and H. R. Thornton[3]

Abstract

Edmonton raw milks and creams contain important numbers of bacteria capable of growing at temperatures of 10.5°C. and 4.5°C. The major source of these bacteria is nonsterile utensils. The psychrophile counts parallel rather closely but probably are not included in the Standard plate counts. As the psychrophilic bacteria rarely survive pasteurization but may be introduced into the pasteurized product by nonsterile plant equipment, their presence in freshly pasteurized milk indicates inefficient plant sanitation.

Introduction

Dairy science is not devoid of reported studies of certain specific defects of dairy products caused by microorganisms capable of growth at low temperatures. But there are few reports concerned with the general occurrence, distribution, and types of psychrophilic dairy bacteria. Such bacteria might be expected to be favored in a northern climate. The present almost universal practice of long farm storage of churning creams and the rapidly increasing tendency to hold pasteurized milk for long periods at low temperatures before consumption emphasize the growing importance of investigations of this type of microorganism.

The present series represents a study of the psychrophilic bacteria in the milks and creams of this area, particularly with respect to numbers, mode of entrance into the product, relation to pasteurization and flavor defects, and some of the characteristics of the organisms.

There is as yet no universally recognized definition of psychrophiles and, thus, the expressions obligate psychrophile and facultative psychrophile have limited meaning. As the bacteria discussed herein grew at 10.5°C. or below and were inhibited at 35.5°C., they are designated as psychrophiles for purposes of report.

Historical

Jezeski and Macy (2) found 20°C. to be superior to 8°C. for growing psychrophiles from water and butter and 21 days at 3°–5°C. gave Thomas and Chandra Sekhar (4) higher counts than did either 7 or 14 days. Burgwald and

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[3] *Professor of Dairying.*

Josephson (1) did not regard either the mesophile or the psychrophile count of commercially pasteurized milk as a good criterion of its keeping quality, despite usual deterioration by psychrophiles. A variation of 5°F. was observed by Thornton *et al.* (5) to make a marked difference in the keeping qualities of cream stored in the range 40°–50°F.

A low incidence of heat-resistance among the psychrophilic bacteria has been noted by a number of investigators (2, 3, 4).

Methods

Ten winter milks were collected at a local milk plant, mainly from the raw milk pooling tank, between Jan. 31 and Apr. 3, 1949. Between May 1 and Sept. 31, 23 summer milks from the same source were analyzed. During the latter period the 28 summer creams were obtained from the same dairy but were removed directly from the producers' cans. Twenty of the creams were ordinary sweet cream, while eight, largely of high bacterial content, were for special use.

The samples, taken in sterile containers, were immediately stored at 4.5°C. until plating, which was not over 1.5 hr. later.

Plating was according to Standard Methods for the Examination of Dairy Products, incubation was at 35.5°C. for two days (termed Standard count), or 10.5°C. or 4.5°C. for seven days, and the counts are averages of duplicate or replicate plates.

Results

35.5°C. Counts

The summer milks had higher average Standard counts than had the winter milks as shown in Tables I and II. Earlier investigations by this Department showed that high counts in Edmonton milks and creams delivered at frequent intervals are caused primarily, not by growth of bacteria in the milk, but by growth of bacteria in the milk utensils. Therefore, the majority of these bac-

TABLE I

PLATE COUNTS OF WINTER MILKS
(in thousands)

Date	35.5°C. count	4.5°C. count
Jan. 31	220	6
Feb. 6	246	8
Feb. 14	322	12
Feb. 21	294	5
Mar. 1	424	120
Mar. 7	98	19
Mar. 14	30	13
Mar. 21	174	30
Mar. 28	104	8
Apr. 4	164	10

TABLE II

PLATE COUNTS OF SUMMER MILKS
(in thousands)

Date	35.5°C. count	10.5°C. count	4.5°C. count
May 16	170	....	190
May 23	95	....	4
May 30	1140	....	190
June 7	190	180	0.5
June 13	303	260	4
June 16	146	93	2
June 21	1430	4000	290
June 24	240	184	4
June 27	207	208	4
June 30	176	155	8
July 4	183	....	16
July 8	741	720	18
July 11	2500	2000	374
July 14	1980	2110	276
July 18	2010	3370	113
July 21	975	930	153
July 23	668	835	180
July 30	430	600	14
Aug. 8	590	760	119
Aug. 15	425	415	134
Aug. 22	2680	4000	136
Aug. 29	820	1650	246
Sept. 6	160	430	123

teria were introduced into the milks by nonsterile utensils. The very high counts of some of the creams probably reflect considerable growth, as daily delivery of these products is not demanded.

Since, as will be shown in the succeeding paper, 35.5°C. inhibited the growth of all the isolates from these plates, the Standard counts of these milks inadequately express their bacterial contents.

10.5°C. Counts

The 10.5°C. counts expressed as the percentage of the 35.5°C. counts varied from 36% to 350% (Tables I to IV). Probably in no instance is the difference between the two counts outside the experimental error of counting bacteria in dairy products by the plate method. It is apparent that these milks and creams contained many bacteria capable of growth at this temperature.

4.5°C. Counts

The 4.5°C. counts were, with one exception, lower than the standard count but were frequently surprisingly high. The low-temperature counts paralleled those at the higher temperatures remarkably closely. The counts varied from 0 to 111% of the Standard counts.

Seasonal Variation

Not only were the summer milk Standard counts in general higher than the winter milk Standard counts but the 4.5°C. counts of the former were also

TABLE III

PLATE COUNTS OF SUMMER CREAMS
(in thousands)

Date	35.5°C. count	10.5°C. count	4.5°C. count
June 13	2870	3900	95
June 16	570	360	17
June 21	8000	4500	185
June 24	2350	900	6
June 27	1170	895	7
June 30	2100	1100	7
July 4	1720	....	35
July 8	1640	1210	43
July 11	4480	3650	....
July 14	9300	9200	740
July 18	19,300	7000	160
July 21	18,400	18,600	4300
July 24	23,900	25,000	5000
July 30	15,000	43,000	1000
Aug. 8	9100	17,200	1830
Aug. 15	55,000	100,000	15,000
Aug. 22	Crowded	Crowded	....
Aug. 29	....	....	....
Sept. 1	Crowded	98,000	15,500
Sept. 6	5400	7300	1260

TABLE IV

PLATE COUNTS OF SPECIAL SUMMER CREAMS
(in thousands)

Date	35.5°C. count	10.5°C. count	4.5°C. count
July 18	6000	3370	23
July 23	42,000	9000	4000
July 30	1100	1250	1000
July 8	100,000	100,000	20,000
July 15	219,000	234,000	150,000
July 22	52,000	103,000	2000
July 29	46,000	161,000	0
Sept. 6	175,000	243,000	....

generally higher. The explanation probably rests on the fact that farm milk utensils in this area are usually stored at outside atmospheric temperature. The winter temperatures are usually sufficiently low to discourage the development even of psychrophilic populations.

Pasteurization

Pasteurized milk is not unknown to have, not only inordinately high Standard plate counts, but also high psychrophile counts, sometimes equalling and even surpassing the Standard counts. The meager literature suggests that the psychrophiles infrequently survive the heat treatment (2, 3, 4).

This view is supported by the data in Table V, which shows that bacteria capable of growth at 10.5°C. did not survive the commercial pasteurization of four milks and two specialties. The homogenized 10% milk was standardized before pasteurization with cream known to be high in psychrophilic content.

TABLE V

PLATE COUNTS OF COMMERCIALLY PASTEURIZED MILKS
(in thousands)

Sample	Incubation			
	35.5°C. 2 days	26°C. 5 days	21.5°C. 5 days	10.5°C. 13 days
1	20	41	37	0
2	25	43	36	0
3	23	41	37	0
4	36	44	38	0
Homogenized milk	16	26	25	0
Homogenized 10% m ilk	26	44	48	0

Moreover, spore-forming psychrophiles were not observed at any time during this study and only four of 722 psychrophilic isolates survived laboratory pasteurization.

There appears to be justification, therefore, for the conclusion that non-sterile plant equipment is responsible for the majority of psychrophiles in freshly pasteurized milk as recently pointed out by Rogick and Burgwald (3).

Discussion

It is widely recognized that the Standard plate count of milk is subject to great variability and may frequently be misleading. There is no equivalent information relative to the enumeration of milk psychrophiles.

Wolochow *et al.* (6) using two media found 10–15°C. plate counts of water to be disturbingly variable and inaccurate and directed attention "to the inadequacy of either medium as a single general measure of total numbers". There is no known reason to regard milk psychrophile counts differently. The exceedingly variable ratios of the low temperature counts to the Standard counts in the present study lend support to this view.

Not a single culture of the 722 isolations reported in the second paper of this series was able to grow at 35.5°C. Therefore, it is highly improbable that the Standard plate counts include many psychrophilic bacteria and are, to this extent at least, underestimates of bacterial populations.

The two lower temperatures for plate incubation were chosen in this study because 50°F. (10°C.) is almost universally recommended as the temperature above which milk and cream should not be stored, while cooling and storage

temperatures below 45°F. (7.2°C.) and even 40°F. (4.44°C.) are easily attained on the farms of this area. That 50°F. may not fulfill expectations commensurate with the emphasis it has received is intimated in the findings of Thornton *et al.* (5) and others and is suggested again in the present data.

Neither spore-formation nor heat-resistance appears to be characteristic of the milk psychrophiles of this area. Psychrophiles in freshly pasteurized milk, therefore, indicate contact of the pasteurized milk with nonsterile surfaces. The possibility of using the psychrophile count as a measure of equipment sanitation in pasteurizing plants is suggested by these results and deserves further investigation.

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PSYCHROPHILIC BACTERIA IN EDMONTON MILK AND CREAM

II. KINDS[1]

By I. E. Erdman[2] and H. R. Thornton[3]

Abstract

The bacterial plates discussed in the preceding paper were the source of 722 isolations of psychrophiles. The cultures, when grouped according to morphology, Gram reaction and certain cultural and biochemical characteristics, were found to be of a more heterogeneous nature than was expected. Seasonal variation was not impressive but increased incubation temperature multiplied the heterogeneity. Thus, storage of milk and cream at temperatures well below 10°C. has qualitative as well as quantitative advantages. Identification was made of 190 cultures, which fell in the following genera with decreasing frequency:—*Pseudomonas*, *Lactobacillus*, *Streptococcus* (lactic acid streptococci), *Aerobacter*, *Flavobacterium*, and *Escherichia*, while there was one culture of *Alcaligenes viscosus*. No sporeformers were encountered and no culture grew at 35.5°C. Only four (all Gram-negative cocci) of the 722 isolates survived laboratory pasteurization. All isolates produced flavor defects in skim milk with stale and oxidized, sour, and bitter predominating. An unusual bitter flavor was found to be caused by certain strains of the genus *Pseudomonas*.

Introduction

Isolations were made from colonies on the plates used for counting the psychrophilic bacteria in the milks and creams reported in the preceding paper (1). The pure cultures so obtained were studied as to morphology and certain other characteristics and segregated into groups and in some cases genera.

Historical

Most studies of dairy psychrophiles have involved too few samples or isolates to assure representativeness. Of 41 psychrophilic isolations made by Jezeski and Macy (3) from 20 butters and 16 waters, 28 were *Pseudomonas*, 6 *Alcaligenes*, 5 *Flavobacterium*, 1 *Achromobacter*, and 1 yeast. Six of these cultures survived laboratory pasteurization in skim milk at 150°F. for 30 min.

Thomas and Chandra Sekhar (5) found 203 psychrophilic isolates to be Gram-negative rods, except for three yellow micrococci. The experimental technique, however, was one which would seem unlikely to discourage interference by mesophiles capable of slow growth at low temperatures.

In the study of Rogick and Burgwald (4) most of the psychrophiles were cocci and nonsporing rods and none was thermoduric.

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Contribution from the Department of Dairying, University of Alberta, Edmonton, Alta.
The data contained herein are taken from a thesis submitted by the senior author to the University of Alberta in partial fulfilment of the requirements for the degree of Master of Science.
This investigation was supported by a grant from the National Research Council of Canada. Issued as Paper No. 262 of the Canadian Committee on Food Preservation.
[2] *Graduate Assistant.*
[3] *Professor of Dairying.*

Methods

Immediately following the counting of the 10.5°C. and 4.5°C. plates as previously reported, colonies were fished onto TGEM agar slopes. The number of isolations per plate varied from a few to all the colonies on the plate and averaged 12.4 per sample.

Gram stains were made from these agar slope cultures after incubation at 12°C. for 24 to 36 hr. Inoculation was then made into four differential media, which, after seven days at 12°C., were observed for reaction in litmus milk, fluorescence in the asparagin broth of Georgia and Poe (2) under ultraviolet light, gelatin liquefaction, and flavor production in skim milk.

Incubation temperatures did not at any time exceed 12°C., except for the determination of the growth temperature range of the pure cultures, and incubation times were not extended beyond seven days in order to minimize confusion with mesophiles.

The identification of the lactic acid streptococci and *Lactobacillus* was based on morphology, Gram reaction, and reaction in litmus milk. The coliforms were recognized as such by the usual methods used in water and dairy bacteriology and were generically identified by the Imvic group of tests and on eosin methylene blue agar.

Experimental

Temperature

All cultures grew at 10.5°C. and 27°C. but none at 35.5°C. Only four of the 722 cultures survived careful laboratory pasteurization in skim milk at 142°F. for 30 min. These were all Gram-negative nonfluorescing cocci which did not liquefy gelatin. They produced sour, stale, and whey flavors in skim milk and were of varied reaction in litmus milk, one culture giving an acid reaction, one slightly alkaline, and two unchanged.

Morphology

Of the total 722 isolations made between Jan. 31 and Sept. 31, 1949, 45% were rods and 55% were coccus forms. Table I would indicate a seasonal vari-

TABLE I

MORPHOLOGY AND GRAM REACTION OF 722 CULTURES

Product	Number of cultures	Gram-negative		Gram-positive	
		Rods, %	Cocci, %	Rods, %	Cocci, %
Summer milk	314	29	32	15	24
Summer cream	252	25	41	12	22
Total summer isolates	566	27	36	13	23
Winter milk	156	45	28	14	13
Totals	722	31	34	14	21

ation with rods predominating in the winter and cocci in the summer but this may be an artifact. Spore formation was not observed at any time throughout the investigation.

Gram Reaction

Of the 722 cultures 65% were Gram-negative and there was little seasonal variation. Gram-negativeness was characteristic of 70% of the rods and, surprisingly, 62% of the cocci. Among the Gram-positive bacteria the coccus forms appeared to predominate.

Litmus milk

Based on litmus milk reaction, 97% of the 4.5°C. winter and 82% of the 4.5°C. summer isolates fell into the four classes, acid, acid proteolytic, unchanged, and alkaline proteolytic. A further breakdown reveals that 15% of the winter cultures were acid, 17% acid proteolytic, 61% unchanged, and 4% alkaline proteolytic, while the equivalent figures were 6% and 17%, 2% and 9%, 36% and 34%, and 37% and 22% for the summer milks and creams respectively. There was an increase in the alkaline proteolytics in the summer samples, coupled with a decrease in the acid proteolytic and unchanged classes. In general there was a tendency for greater heterogeneity among the summer isolates.

A somewhat greater heterogeneity was also noted among the 10.5°C. isolations from both the milks and the creams. Thus, a 6°C. increase in temperature gives encouragement, not only to increased numbers as previously shown, but also to increased kinds.

Gelatin Liquefaction

Gelatin was liquefied by 35% of the total cultures, 63% of the Gram-negative rods, 14% of the Gram-negative cocci, 31% of the Gram-positive rods, and 29% of the Gram-positive cocci. This confirms the litmus milk results that the milks and creams of this area contain many proteolytic bacteria capable of growth at low temperatures.

Fluorescence

Fluorescence was exhibited by 26% of the total cultures, 62% of the Gram-negative rods, 14% of the Gram-negative cocci, 8% of the Gram-positive rods, and 3% of the Gram-positive cocci.

Flavor Defects

Without exception all cultures produced flavor defects in skim milk as set forth in Table II. Stale and oxidized flavors were noticed in 274 (38%) cultures, various sour flavors in 168 (23%), and bitter flavors in 133 (18%). Other off-flavors noted included slight or incipient rancidity, putrefaction, fermentation, and whey.

Identification

The identification of most of the 722 cultures was not within the scope of this study. Nevertheless, some species and some genera of bacteria are rather

TABLE II

THE NUMBER OF CULTURES EXHIBITING OFF-FLAVORS IN SKIM MILK

Gram reaction	Morphology	Total isolates	Stale and oxidized	Sour	Bitter	Putrefactive	Incipient rancidity	Fermentation	Whey
Negative	Rods	224	53	22	68	33	33	15	0
	Cocci	246	153	24	28	4	20	17	0
Positive	Rods	99	28	39	17	11	0	4	0
	Cocci	153	40	83	20	2	6	0	2
Total		722	274	168	133	50	59	36	2

easily recognized. All the isolates were examined by rather simple methods for certain characteristics which permitted the identification of 190 cultures.

Pseudomonas spp. accounted for 16.7% of all cultures and 54% of the Gram-negative rods. Their frequency of isolation from the summer samples was almost double that from the winter milks.

Of the 20 lactic acid streptococci, 17 produced the typical *S. lactis* reaction in litmus milk, while three failed to coagulate the medium in seven days at 12°C. One culture was isolated from winter milks, while three were summer isolates at 4.5°C. and 16 at 10.5°C. Summer samples at 10.5°C. were the source of all 32 *Lactobacillus* cultures except one. It would seem that the Edmonton climate has not succeeded in overcoming the characteristic reluctance of these genera to grow at low temperatures.

The coliforms did not seem to be affected by season but their low incidence is interesting, the genus *Escherichia* accounting for only two cultures and *Aerobacter* for eight. Six yellow Gram-negative rods were isolated in these studies and all belong to the genus *Flavobacterium*. Only one ropy culture was observed in the entire study and it proved to be *Alcaligenes viscosus*.

An Unusual Type of Bitterness

Various forms of bitterness occur frequently in the dairy products of this area, particularly in churning creams. Thornton *et al.* (6) were impressed by a peculiar and striking bitterness which developed in some of their creams of low bacterial content which were held for long periods at low temperatures. This was a bitterness suggestive of the alkaloids rather than of peptones, fatty acids, or aldehydes. It is probable that the same type of bitterness is occasionally encountered in the commercial grading of churning creams.

In an endeavor to find the cause of this type of bitterness, 20 commercial milks and creams, not otherwise reported, were incubated at 4.5°C. for 15 days. The typical peculiar bitterness developed in two creams and four milks, all of low initial bacterial content. Twelve cultures were isolated, all of which grew between 4.5°C. and 32°C. but none at 37°C. Three of these cultures did not produce bitterness in skim milk and were generically identified as *Bacterium*. Three of the 12 isolates could not be classified beyond the Tribe Pseudo-

monadeae and they produced a slight but not typical bitterness in skim milk culture.

The remaining six isolates gave the typical bitter flavor in skim milk and were given the laboratory designations Groups A and B. The three cultures in Group A differed only slightly from *P. fluorescens* and were considered variants of that species. The three cultures in Group B differed from Group A as *P. viscosus* differs from *P. fluorescens* but varied somewhat from *P. viscosus*.

In the nine cultures of Pseudomonadeae the bitter flavor became increasingly pronounced as *P. fluorescens* was approached in characteristics.

Discussion

In general dairy practices 10°C. (50°F.) has assumed a place of importance as a storage temperature for milk and cream. It was previously shown that there are distinct advantages to even lower storage temperatures for inhibiting bacterial growth and in lengthening the period of usability of the product (6). A further advantage is now evident in that, because temperature has a selective action, the flora becomes increasingly simplified as temperature is lowered. It is significant that every culture isolated in this study exhibited undesirable flavor characteristics. Simplification of the flora, both qualitatively and quantitatively, will also simplify the off-flavor problem.

At various times, in various ways, and for various purposes several thousand isolations have been made within the psychrophilic range by this laboratory. The high incidence of pseudomonads among these isolates has been striking. At times it has amounted to over 30% of the isolations. In the majority of cases flavor defects were under scrutiny and it is significant that every culture of this type which has been examined exhibited undesirable flavor characteristics. The group is undoubtedly responsible for a heterogeneity of flavor defects and deserves further research attention.

This study revealed a greater heterogeneity of types among the psychrophilic bacteria in the milks and creams of this area than was expected from the literature on the subject and from probable climatic influences. No explanation is offered for this observation. Type simplification followed temperature decrease but, on the other hand, type heterogeneity was strikingly greater than that reported from more temperate zones.

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EXTRACTION OF POLYHYDROXY COMPOUNDS FROM DILUTE AQUEOUS SOLUTIONS BY CYCLIC ACETAL FORMATION

I. AN INVESTIGATION OF THE SCOPE OF THE PROCESS[1]

By R. R. Tink[2] and A. C. Neish

Abstract

When a dilute aqueous solution of 2, 3-butanediol is treated with an excess of *n*-butyraldehyde in the presence of dilute hydrochloric acid most of the diol is removed from the aqueous phase and is concentrated in the butyraldehyde phase as the cyclic acetal. Glycerol, sugars, and sugar alcohols may be extracted from aqueous solutions in a similar fashion. The procedure is particularly effective with mannitol, sorbitol, adonitol, dulcitol, and 2, 3-butanediol, a single treatment with one-half volume of butyraldehyde removing 99% of these compounds from water containing them in 4% concentration. The sugars are extracted more slowly and less completely than the sugar alcohols. Considerable differences were found between different sugars, sorbose, and mannose being extracted much more effectively than glucose and galactose. Butyraldehyde gave better extraction than any of the 12 carbonyl compounds tested, cyclohexanone being the next best.

Introduction

A good method for extracting water-soluble organic compounds, such as sugars, sugar alcohols, glycols, and glycerol, from dilute aqueous solutions would be of considerable value in the development of chemurgic processes. Certain important raw materials, such as molasses, waste sulphite liquor, and wood hydrolyzates, contain sugars which are difficult to isolate in a pure state. These crude raw materials can be fermented to produce alcohol which can be readily recovered by distillation, but other important fermentation products, i.e. glycerol and 2, 3-butanediol, are not so easy to obtain pure since they are hygroscopic materials with boiling points higher than that of water.

Solvent extraction is often an economic process for removing materials from aqueous solutions but unfortunately it can not be used effectively for the extraction of sugars or sugar alcohols since the partition coefficients, even with the best solvents, are too unfavorable. However, if some derivative of these compounds could be formed which possessed less affinity for water an efficient extraction might be obtained. This paper describes some experiments on solvent extraction of this type.

When a dilute aqueous solution of a polyhydroxy compound is treated with an excess of an immiscible aldehyde, such as butyraldehyde, a diphasic liquid

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Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Sask. Issued as paper No. 110 on the Industrial Utilization of Wastes and Surpluses, and as N.R.C. No. 2383.
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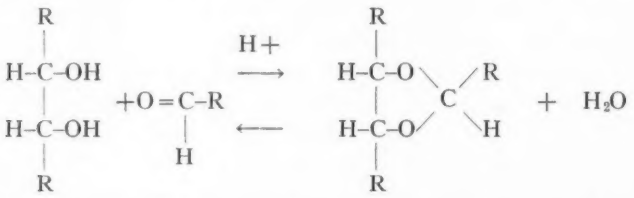
system is obtained. If an acid is present cyclic acetal formation ensues by the following well known reaction.



The acetal is much less soluble in water than the polyhydroxy compound owing to the blocking of hydroxyl groups and increase of molecular weight, and hence is extracted fairly completely into the butyraldehyde phase. The amount which can be extracted in one operation depends primarily on the equilibrium point. The extraction is not as rapid as a conventional solvent extraction process since its rate is limited to that of a chemical reaction. Kinetic equations for this type of extraction are given in the next paper of this series (4). This paper reports data on the rate and extent of extraction of some sugars, glycols, and sugar alcohols using various carbonyl compounds.

This type of extraction has not received much attention. However, Senkus (3) has devised a process for recovering 2, 3-butanediol which is based on formation of the formaldehyde derivative. In this process, distillation, rather than solvent extraction, is used to remove the cyclic formal from the aqueous solution.

Experimental

Materials

The best grades of chemicals available commercially were used. All liquid chemicals were redistilled except glycerol and ethanediol. The sugars and sugar alcohols were chiefly Difco and Eastman products. Their melting points and optical rotations were measured and found to check fairly well with those recorded in the literature.

Determination of Polyhydroxy Compounds and their Acetals

All polyhydroxy compounds were estimated by oxidation with periodic acid, the amount of periodic acid reduced being determined. When cyclic acetals were present the mixture was first subjected to acid hydrolysis and a measurement of the total polyhydroxy compound, free and combined, thus obtained. The analytical procedure, which is based on that of Rappaport, Reifer, and Weinmann (2) is as follows. An aliquot containing polyhydroxy compound equivalent to 1.0 mgm. of glycerol, or less, is mixed with 5 ml. of 0.4 *N* sulphuric acid and 5 ml. of 0.005 *M* potassium periodate in a 125 ml. Erlenmeyer flask. The walls of the flask are rinsed with distilled water and the solution is again mixed thoroughly and allowed to stand one hour at room temperature. Then 10 ml. of 0.5 *M* disodium hydrogen phosphate is added, and, after

thorough mixing, an excess of crystalline potassium iodide. The iodine released is equivalent to the periodate remaining in the solution since iodate will not oxidize iodide in the range pH 5–pH 7 (2). The iodine is titrated with 0.001 *N* sodium thiosulphate (freshly prepared by dilution of an 0.1 *N* solution), using starch as an indicator. The difference between titrations of a blank and the sample is equivalent to the periodate reduced by the polyhydroxy compound. The amount of polyol is calculated from the known stoichiometric reactions.

When an acetal is present the sample is heated with the acid in a pressure bottle immersed in boiling water for one hour. After cooling the periodate is added and the analysis completed as above.

Determination of Sugars and Their Acetals

The sugars were determined by the color reaction with anthrone, as suggested by Morris (1). Five ml. of a solution containing less than 150γ of sugar was pipetted into a colorimeter tube 25 mm. in diameter. Ten milliliters of the anthrone reagent (2 gm. anthrone in 1 liter of 95% sulphuric acid) was added, using a syringe to obtain thorough reproducible mixing. After the mixture had stood 10 to 15 min. the per cent transmission was read at 640 mμ, using a Coleman Model 6 spectrophotometer. Standard solutions of the sugar in question were run with the test samples in order to get a calibration curve, and the amount of sugar present in the unknown read from the graph thus obtained. The conditions for the development of color are sufficient to hydrolyze the acetals (even cellulose is hydrolyzed quantitatively) hence both the free and combined sugars are determined. Carbonyl compounds give a red color with this reagent but it is quite feasible to measure the blue color due to sugars in its presence. In order to increase the accuracy of the method sufficient butyraldehyde was added to standard solutions to approximate the amount in the test samples so that small variations in the amount of carbonyl compound would not cause much error. At worst, a 13% change in *n*-butyraldehyde concentration made a 2% error in the amount of sugar found. The procedure above applies to hexoses, but the same technique was used for the determination of arabinose which gives considerably less color and thus requires the use of stronger standard solutions.

Determination of the Efficiency and Rate of Extraction of a Polyhydroxy Compound

The mixture of water, polyhydroxy compound, acid, and immiscible carbonyl compound was stirred vigorously in a reaction bottle at a constant temperature. The stirring was stopped at definite time intervals, the phases allowed to separate, and a small sample of the aqueous phase taken for analysis. The stirrer was shut off for approximately 20 sec. each time a sample was taken. When equilibrium was reached, a measurement of the relative volumes of the two phases was made and the concentration of the polyhydroxy compound in both phases was measured. It was assumed that the short interruptions in the stirring would not affect the rate of removal of the polyhydroxy compound since it did not with glycerol (4).

The rate of the extraction can also be calculated from the data obtained as described above. The logarithm of the difference between the amount of polyhydroxy compound in the aqueous phase (at any given time) and its concentration at equilibrium when plotted against time gives a straight line (4).

Recovery of D-(*levo*)-*2, 3-Butanediol from Dilute Aqueous Solution*

Two hundred ml. of an aqueous 2, 3-butanediol solution (4.00%) containing hydrochloric acid (0.387 *N*) was agitated for three hours with 10 ml. of *n*-butyraldehyde. The aldehyde phase was washed with dilute sodium bicarbonate and fractionally distilled. Water was added to bring the acetal over as the azeotrope (b.p. 95°C.) after separation of the aldehyde. The yield of pure acetal (b.p. 147°C. 719 mm., N_D^{25} 1.4074, $(\alpha)_D^{25}$-14.2°) was 12.35 gm. or 94.1% of the theoretical, assuming all the diol could be extracted. The acetal was hydrolyzed at 100°C. for one hour with a slight excess of water in the presence of 15 gm. of a cation exchange resin (Amberlite IR-105) in the acid form. The butyraldehyde released by hydrolysis was continually removed by distillation through a fractionating column so the reaction would go to completion. The resin was filtered off and the hydrolyzate distilled under reduced pressure to give 7.12 gm. of the pure 2, 3-butanediol (89.2% overall recovery).

Recovery of D-*Mannitol from Dilute Aqueous Solution*

An aqueous solution of mannitol (4.00%) was treated under the conditions described above for 2, 3-butanediol. After removal of the butyraldehyde, the acetal was obtained as a gel-like substance, probably consisting of a mixture of compounds. Hydrolysis gave 7.24 gm. of pure crystalline mannitol (90.8% of that originally put in the aqueous solution).

Results

A number of liquid carbonyl compounds were compared for their efficiency in extracting D-(*levo*)-2, 3-butanediol from water. The compounds used, the conditions under which the experiments were run, and the results obtained are shown in Table I. These aldehydes and ketones were selected because they are the most readily available of liquid carbonyl compounds immiscible with water. They are arranged in decreasing order of effectiveness. Large differences between these compounds were found, *n*-butyraldehyde and cyclohexanone being much better than the others.

n-Butyraldehyde was selected for further study, on the basis of the above results, and its efficiency in extracting a number of polyols and sugars determined. The results are shown in Table II. The glycols and sugar alcohols are shown first, in decreasing order of effectiveness of their extraction and then the sugars in the same order. The sugar alcohols and 2, 3-butanediol are extracted almost completely. The distribution coefficients for glycerol and ethanediol are favorable for their extraction but are considerably lower than those of the 2, 3-butanediol and the sugar alcohols and somewhat lower than those for some of the sugars. The differences between the completeness of extraction of the

TABLE I

COMPARISON OF CARBONYL COMPOUNDS FOR EFFICIENCY IN EXTRACTING D-(*levo*)-2, 3-BUTANEDIOL FROM WATER

Each system contained 52.6% water, 16.4% of D-(*levo*)-2, 3-butanediol, 1% *p*-toluenesulphonic acid, and 30% of the carbonyl compound (by weight). The reaction was allowed to run at 25°C. until equilibrium was reached

Carbonyl compound	Concentration of butanediol* at equilibrium		Distribution coefficient**
	Aqueous phase (%)	Carbonyl phase (%)	
n-Butyraldehyde	0.6	35.6	79
Cyclohexanone	2.0	42.0	21
Benzaldehyde	13.0	34.4	2.65
Butanone	14.4	37.0	2.57
3-Methylbutanone-2	15.4	24.4	1.58
Pentanone-3	17.1	26.0	1.52
Mesityl oxide	21.3	14.0	0.66
2-Ethylhexaldehyde	24.3	7.3	0.30
Acetophenone	24.7	5.2	0.21
2, 4-Dimethylpentanone-3	25.5	3.5	0.14
Heptanone	24.6	2.8	0.11
4-Methylpentanone-2	24.0	1.0	0.04

* *The sum of the concentrations in the two phases is not constant for different systems because of variation in the relative phase volumes due to the differing solubilities of the carbonyl compounds in water.*

** *Ratio of concentration of the diol (free and combined) in the solvent phase to that in the aqueous phase.*

TABLE II

EXTRACTION OF GLYCOLS, SUGAR ALCOHOLS, AND SUGARS FROM WATER BY *n*-BUTYRALDEHYDE

Each system contained 4 gm. of polyol or sugar, 100 ml. of dilute hydrochloric acid, and 50 ml. of *n*-butyraldehyde. The hydrochloric acid was 1.78 *N* (for sugars) or 0.378 *N* (for glycols and sugar alcohols). The reaction was run at 30°C. until equilibrium was reached

Polyhydroxy compound	% in aqueous phase at equilibrium	Distribution coefficient	Rate constant,* $sec.^{-1} \times 10^4$	Half-time** (min.)	Material balance, %
D-Sorbitol	0.01	788	12.0	6.0	96.5
Adonitol	0.02	461	6.87	11.5	100.4
Dulcitol	0.02	461	11.4	8.0	101.5
D-(*levo*)-2, 3-Butanediol	0.04	204	22	0.9	97.0
D-Mannitol	0.05	189	8.12	11.0	99.5
Glycerol	0.73	8.29	15.7	5.0	96.4
Ethanediol	1.21	5.89	16.6	4.8	104.1
L-Sorbose	0.27	22.9	1.16	63	80.0
D-Mannose	0.62	11.6	2.77	48	95
D-Fructose	0.75	7.0	2.50	49	76.7
L-Arabinose	2.56	1.63	....	....	105.0
D-Glucose	2.32	1.27	0.92	125	95.0
D-Rhamnose	3.05	0.606	....	....	95.1
D-Galactose	3.50	0.400	....	....	100.5

* *Calculated from experimental data using equation given in next paper of this series (4).*

** *Time required for process to go half way to the equilibrium point.*

various sugars are quite interesting. Mannose and the ketoses are extracted much more readily than glucose or galactose.

The time required for the extraction is also an important factor in this type of process. A comparison of the rates of extraction of the sugars and polyols was made (Columns 4 and 5, Table II). The glycols and sugar alcohols, particularly 2, 3-butanediol are extracted more rapidly than the sugars even though less catalyst was used. The ketoses are partially destroyed under the conditions used, judging from the low recovery of material, while the aldoses are affected only slightly.

Because of interest in the possibility of recovering pure sugars from crude aqueous solutions the behavior of glucose and fructose was examined further. The addition of alcohols was tried, since it was thought that formation of the glycosides might aid extraction. Cyclohexanone and benzaldehyde were compared with butyraldehyde. The results are shown in Table III. *n*-Butyraldehyde

TABLE III

EXTRACTION OF GLUCOSE AND FRUCTOSE FROM WATER

Each system contained 4 gm. of the sugar, 100 ml. of 1.78 *N* hydrochloric acid, 25 ml. of the alcohol (when added), and 50 ml. of the carbonyl compound; the reaction was run at 30°C. until equilibrium was attained.

Carbonyl compound	Alcohol	% sugar in aqueous phase at equilibrium		Distribution coefficient		Rate constant, $sec.^{-1} \times 10^4$		Half-time (min.)	
		Glucose	Fructose	Glucose	Fructose	Glucose	Fructose	Glucose	Fructose
n-Butyraldehyde	....	2.32	0.75	1.27	7.00	0.92	2.50	125	49
n-Butyraldehyde	Methanol	2.00	1.00	1.60	3.90	1.12	2.90	89	40
n-Butyraldehyde	*n*-Butanol	2.25	0.97	1.13	3.71	0.37	....	193	....
Cyclohexanone	....	3.60	2.35	0.10	0.62	....	....	....	....
Cyclohexanone	Methanol	2.75	2.85	0.22	0.35	....	....	....	....
Benzaldehyde	....	3.70	3.25	0.07	0.07	....	....	....	....

NOTE: *About 95–100% of the glucose was accounted for but only 72–77% of the fructose.*

is more efficient than the other carbonyl compounds for the extraction of these sugars. Addition of methanol improved the extraction of glucose somewhat while butanol was unfavorable. Both alcohols decreased the efficiency of the extraction of fructose.

The product of the extraction in this type of process is the acetal of the polyhydroxy compound. After separation from the excess of butyraldehyde it may be hydrolyzed by a small amount of dilute acid and the hydroxy compound recovered by distillation or crystallization, depending on its properties. Experiments with 2, 3-butanediol and mannitol showed that they could be recovered from aqueous solutions in overall yields of about 90% (see Experimental Section).

Discussion

The data reported in this paper suggest that *n*-butyraldehyde might be a useful solvent for the recovery of polyhydroxy compounds from dilute aqueous solutions, provided conditions are favorable for cyclic acetal formation. It should be possible to separate these compounds from other water-soluble compounds, such as salts, which will not react with the aldehyde. Since there are considerable differences between different polyhydroxy compounds it may even be possible to resolve mixtures by use of a counter-current technique. The large differences between sugars and sugar alcohols, both in rate and degree of extraction, indicate that these two classes of compounds could be separated and even mixtures of certain sugars such as mannose and glucose might be resolvable. The ease of extraction of sugar alcohols is rather surprising considering that they have five or six hydroxyl groups in each molecule. Presumably the sugar alcohols react more rapidly than sugars because their molecules can twist themselves into positions favorable for cyclic acetal formation more readily than the sugar molecules, which are made less flexible by formation of pyranose or furanose rings.

Extraction of polyhydroxy compounds by butyraldehyde might be useful in a number of industrial processes. One example worth considering is the recovery of glycerol and 2, 3-butanediol from fermentation solutions. If these compounds were extracted from the dilute solutions the necessity of evaporating large quantities of water would be avoided. At the same time they could be more readily purified by distillation of the acetals because of the lower boiling points. Unfortunately the hydrolysis of the acetals to recover the 2,3-butanediol or glycerol would add considerably to the cost of the process. Theoretically all the butyraldehyde is recoverable but there are certain to be small losses due to oxidation and polymerization which might be sufficient to render such a process uneconomic. If the acetals themselves could be used directly with advantage in some reaction, such as preparation of alkyd resins, this method of recovery would be more attractive.

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EXTRACTION OF POLYHYDROXY COMPOUNDS FROM DILUTE AQUEOUS SOLUTIONS BY CYCLIC ACETAL FORMATION

II. THE BATCH EXTRACTION OF GLYCEROL[1]

By R. R. Tink,[2] E. Y. Spencer, and J. M. Roxburgh

Abstract

The amount and rate of extraction of glycerol from dilute aqueous solutions by acetal formation have been studied. Of 12 carbonyl compounds, *n*-butyraldehyde gives the most favorable distribution, and further studies were made with it only. The amount of free and combined glycerol remaining in the aqueous phase at equilibrium is proportional to the initial glycerol concentration, and increases with increasing temperature. The reciprocal of the residual glycerol concentration is a linear function of the ratio of *n*-butyraldehyde to water. The rate of extraction increases linearly with mineral acid concentration. The same linear increase in rate is observed with increasing amounts of cation exchange resin as catalyst, but grinding the resin to a smaller size increases the catalytic activity to a much larger extent than the increase in exchange capacity so produced would indicate. The rate of extraction increases with increasing temperature and with increasing ratio of *n*-butyraldehyde to water.

Introduction

Regardless of the primary source, glycerol must always be recovered from aqueous solution as one step in its manufacture. Evaporation and distillation under reduced pressure is the usual method, but the low volatility of glycerol and the large amounts of water associated with it make this process comparatively expensive. Solvent extraction is not suitable in this case as the distribution coefficient between water and any known immiscible solvent is unfavorable.

In a prior paper in this series (5) it was shown that polyhydroxy compounds are efficiently extracted from dilute aqueous solutions by an excess of an immiscible carbonyl compound. The cyclic acetal is formed and extracted into the carbonyl phase which acts as solvent as well as reactant. The present study covers the choice of carbonyl compound to be used, and the kinetics of the reaction, with glycerol.

Three useful relations can be derived for the kinetic study of this reaction (6):—

$$\frac{G_0}{G_e} = fn + L \qquad (1)$$

$$-Ca(t_1 - t_2) = ln\,\frac{G_1 - G_e}{G_2 - G_e} \qquad (2)$$

and $$a = bn + a \qquad (3)$$

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Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Sask., and the University of Saskatchewan, Saskatoon Sask. Issued as Paper No. 111 on the Industrial Utilization of Wastes and Surpluses, and as N.R.C. No. 2384.
[2] *Present address, McGill University, Montreal, Que.*

where G_0, G_1, G_2, and G_e are glycerol concentrations in the aqueous phase initially, at times t_1 and t_2, and at equilibrium, respectively.

n is the ratio of carbonyl compound to water,
$C\alpha$ is *defined* as the rate constant for the extraction,
C is the effective concentration of hydrogen ions, and
f, L, b, and a, are constants.

These equations require the assumptions that: (*a*) the reaction is bimolecular, reversible, and catalyzed by hydrogen ions; (*b*) the distribution coefficients for acetal and glycerol are constants; (*c*) glycerol is almost entirely in the aqueous phase; and (*d*) the volume change in a phase during the course of the reaction has a negligible effect on the concentration of the major component of that phase.

Experimental

Materials

All carbonyl compounds were redistilled and midfractions of correct index of refraction and boiling point used (2). Glycerol and hydrochloric acid were the best analytical grade. The ion exchange resin used was Duolite C3, obtained from the Chemical Process Company, San Francisco, Calif. It was sized, regenerated by soaking in 5% hydrochloric acid, washed until the effluent was neutral, and air-dried. The exchange capacity was determined by titration in the presence of an excess of potassium chloride with methyl purple indicator.

Determination of Glycerol

Glycerol was determined by the periodate oxidation method described previously (5). All samples containing acetal were hydrolyzed for one hour with acid before addition of the periodate, so that total glycerol, combined and free, was determined. When glycerol was to be determined in the presence of acetal, acid, phosphate buffer, and periodate were added to the sample simultaneously and the excess periodate determined after 15 min. at room temperature. Under these conditions acetal does not interfere appreciably and over 98% of the glycerol reacts.

Determination of the Equilibrium Constant

Weighed amounts of either a carbonyl compound and glycerol or of an acetal were added to a weighed amount of slightly acidified water, sufficient to form a homogeneous solution. After two days at constant temperature the concentration of free glycerol and of glycerol plus acetal was determined. In some cases *n*-butyraldehyde was also determined as a check. Equilibrium constants were calculated for the formation of the acetal, and the ratio of free to combined glycerol at equilibrium reported.

Determination of the Efficiency and Rate of Extraction

The mixture of water, glycerol, acid, and carbonyl compound was stirred vigorously in a reaction bottle maintained at constant temperature. Period-

ically, stirring was stopped and a sample taken after about 15 sec. had been allowed for settling. The rate of removal of glycerol from the aqueous phase was not affected by the cessation of stirring for these comparatively short intervals (6). When equilibrium was reached (from two hours to several days, depending on the temperature) the volumes of each phase were measured and the glycerol concentrations determined.

Samples were hydrolyzed for the determination of glycerol, and the total of glycerol and acetal, calculated as glycerol, was used in Equation (1) to determine the rate constant. A slight error is introduced by using the total glycerol instead of free glycerol but more consistent results are obtained.

Results and Discussion

Recovery of Glycerol and the Structure of the Acetal

The acetal of glycerol and *n*-butyraldehyde was prepared under substantially the same conditions as were used in the extraction experiments, and the pure acetal recovered by distillation under reduced pressure (b.p. 118°C./30 mm.). Glycerol was recovered in good yield from the acetal by hydrolysis with a cation exchange resin (Amberlite IR-105), evaporation of the *n*-butyraldehyde and water, and distillation under reduced pressure.

The structure of the acetal is of interest since either a five or six membered ring could be formed (Fig. 1). Methylation of the acetal with methyl iodide

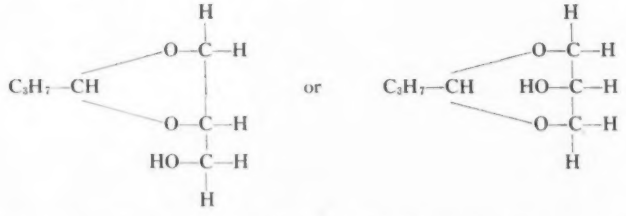


FIG. 1. *Alternative ring structures for the acetal of glycerol and n-butyraldehyde.*

and silver oxide, followed by hydrolysis of the ether extract with dilute hydrochloric acid, gave a compound with boiling point 137°C./40 mm. and refractive index n_D^{25} 1.4422, in good yield. These physical constants correspond to the α-monomethyl ether of glycerol, indicating that the original acetal had the five membered ring structure. None of the β-monomethyl ether of glycerol could be detected. This result agrees qualitatively with work reported by Hann *et al.* (1) on cyclic acetals of the sugar alcohols but is at variance with the results of Hibbert and co-workers (7).

Relative Efficiency of Carbonyl Compounds

Twelve carbonyl compounds were tested for their efficiency in the extraction of glycerol. From Table I, *n*-butyraldehyde is most suitable, and cyclo-

hexanone next best, in agreement with prior work on the extraction of 2,3-butanediol (5). A similar correlation of results has been reported for acyclic acetals (3).

TABLE I

RELATIVE EFFICIENCY OF CARBONYL COMPOUNDS

All systems contained 30.0% carbonyl compound, 49.0% water, 20.0% glycerol, and 1.0% *p*-toluenesulphonic acid. The temperature was 25.0°C.

Carbonyl compound	Concentration of glycerol		Distribution coefficient
	Aqueous phase, gm./100 ml.	Carbonyl phase, gm./100 ml.	
n-Butyraldehyde	10.7	22.6	2.09
Cyclohexanone	20.7	17.6	0.85
Methyl isopropyl ketone	25.6	1.3	0.05
Mesityl oxide	24.4	0.6	0.02
Methyl *n*-amyl ketone	27.2	0.6	0.02
Benzaldehyde	27.6	0.6	0.02
Diisopropyl ketone	27.3	0.5	0.02
2-Ethylhexaldehyde	27.3	0.4	0.01
Acetophenone	27.3	0.4	0.01

Equilibrium Constants

Approximate equilibrium constants for the formation of the cyclic acetals of both *n*-butyraldehyde and cyclohexanone with glycerol are shown in Table II. That for *n*-butyraldehyde was determined at two temperatures and the equilibrium approached from both directions. The significant feature implied in these constants is the ratio of free to combined glycerol in the aqueous layer of a two-phase system at equilibrium. With *n*-butyraldehyde (at 30°C.) over two-thirds of the residual glycerol in this phase is in the form of the acetal, while with cyclohexanone (solubility 2.4 gm. per 100 ml. at 30°C.) less than one-quarter is as acetal.

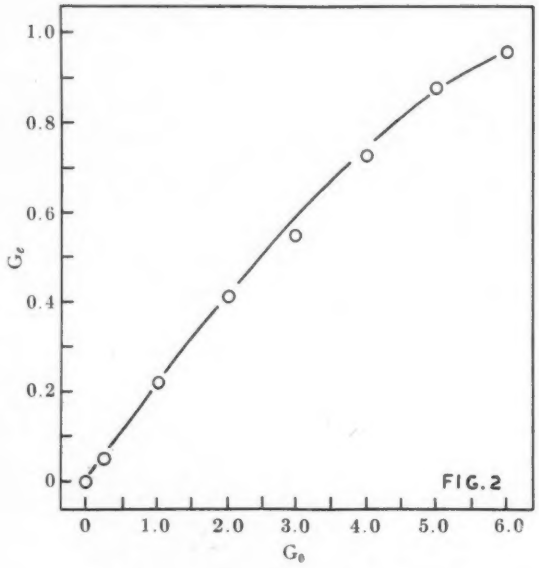
The efficiency of *n*-butyraldehyde in relation to cyclohexanone is therefore even greater than indicated by Table I. The portion of the residual glycerol in the aqueous phase which has reacted to form acetal can probably be recovered by ordinary solvent extraction, although no suitable solvent has been tested as yet.

Kinetics of the Extraction

The effect of initial concentration of glycerol and the ratio of *n*-butyraldehyde to water on the equilibrium glycerol concentration in the aqueous phase is shown in Figs. 2 and 3 respectively. From Fig. 3 an empirical equation corresponding in form to Equation (1) may be calculated as

$$\frac{G_0}{G_e} = 7.8n + 0.9$$

at 30°C. The initial slope of the plot in Fig. 2 agrees with these values for the constants. The effect of temperature on the residual glycerol concentra-



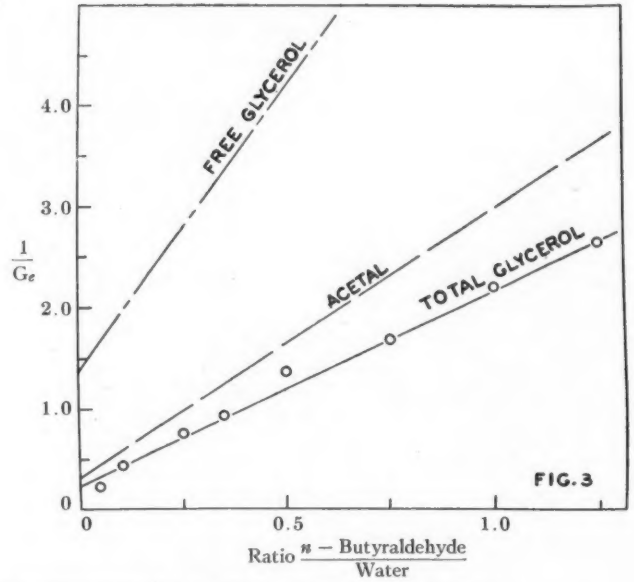
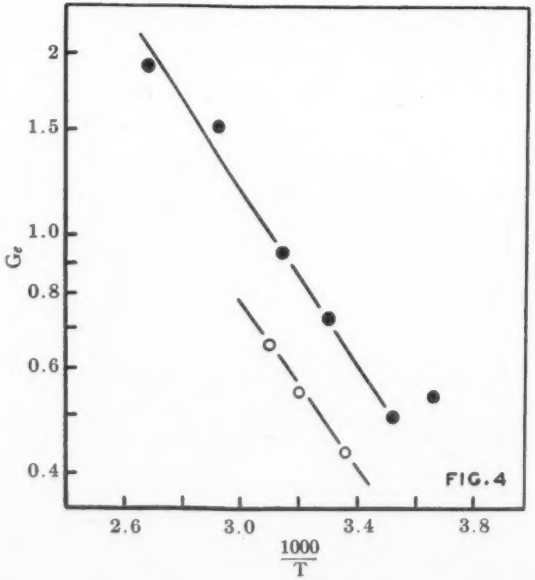


FIG. 2. *The effect of the initial concentration of glycerol (G_0) on the concentration at equilibrium in the aqueous phase (G_e).*
Conditions: *Ratio of carbonyl to water 0.500, 30°C., and 0.378 N with respect to hydrochloric acid. Concentrations in gm. per 100 ml.*
FIG. 3. *Effect of the ratio of n-butyraldehyde to water on the equilibrium concentration of glycerol in the aqueous phase (G_e).*
Conditions: *Initial concentration of glycerol, 4.00%; at 30°C.; and 0.378 N with respect to hydrochloric acid. Concentrations in gm. per 100 ml.*



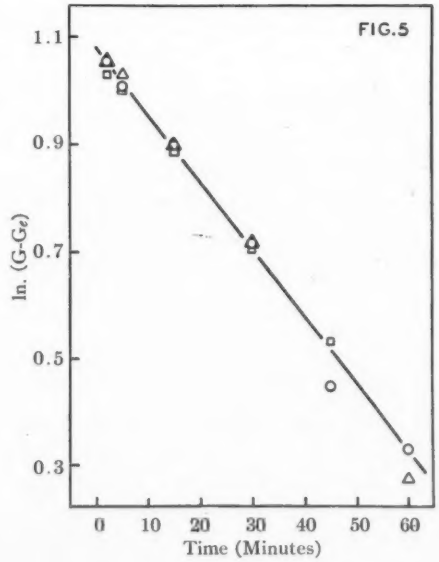
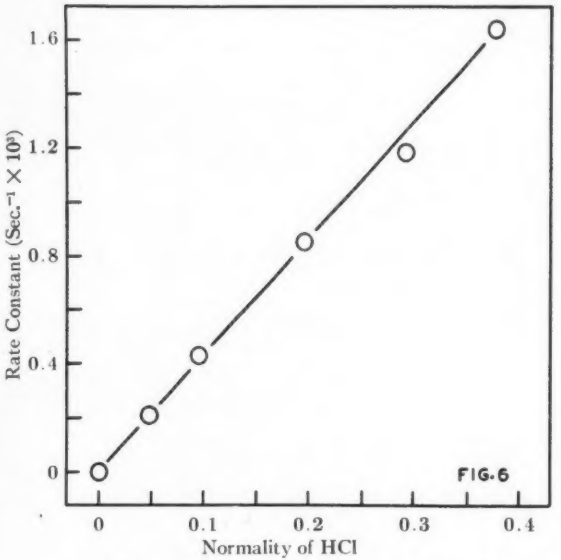


FIG. 4. *Effect of absolute temperature (T) on the equilibrium concentration of glycerol in the aqueous phase (G_e) in gm. per 100 ml.* Conditions: ● = *ratio of n-butyraldehyde to water, 0.500; initial concentration of glycerol, 4.00%; and 0.0489 N with respect to hydrochloric acid.* ○ = *217 ml. n-butyraldehyde; 224 ml. water containing 3.90% glycerol; and 16.4 gm. of Duolite C3, 300-325 mesh, in the acid form.*

FIG. 5. *A typical plot of the change of glycerol concentration in the aqueous phase (G) with time. G_e is the concentration at equilibrium. Concentrations in gm. per 100 ml.*



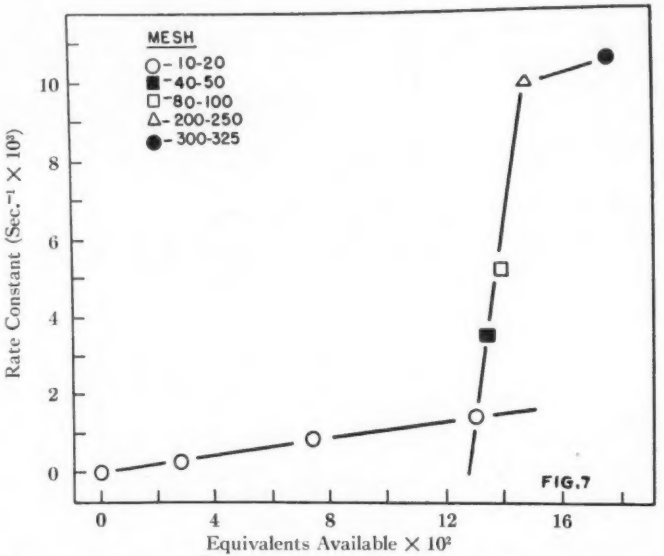
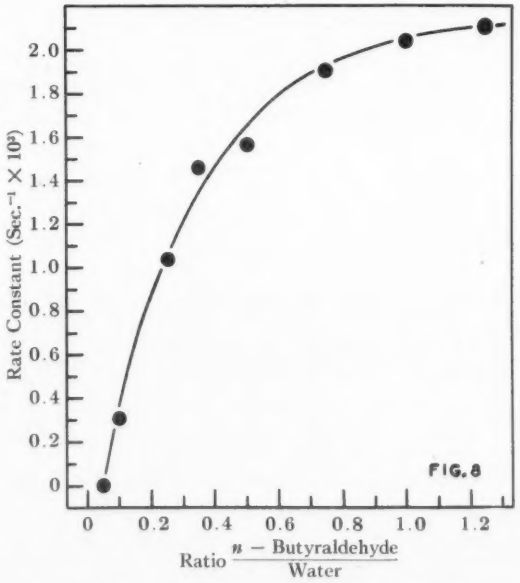


FIG. 6. *Effect of acid concentration on the rate constant. Aqueous phase initially contained 4% glycerol. Ratio of n-butyraldehyde to water, 0.500; at 30°C.*

FIG. 7. *Effect of exchange capacity of cation exchange resin on the rate constant.*
Conditions: *217 ml. n-butyraldehyde, 224 ml. water containing 3.90% glycerol, at 25°C.*



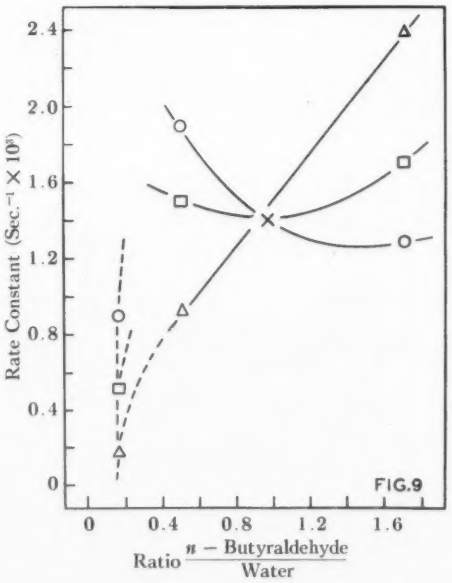


FIG. 8. *The effect of the ratio of n-butyraldehyde to water on the rate constant.*
Conditions: *Aqueous phase contained 4% glycerol, and was 0.378 N with respect to hydrochloric acid, initially 30°C.*
FIG. 9. *Effect of ratio on the rate constant with cation exchange resin. Rate constants recalculated to constant weight of resin per unit volume of:* ○, *aqueous phase;* □, *both phases;* Δ, *carbonyl phase. At 25°C., 3.90% glycerol initially in the aqueous phase.*

TABLE II

EQUILIBRIUM CONSTANTS FOR THE FORMATION OF ACETALS

$$K = \frac{[W]\,[A]}{[G]\,[B]}$$

Carbonyl compound	Initial concentration (M)				Equilibrium concentration (M)				K	Temp. (°C.)
	Carbonyl	Glycerol	Water*	Acetal	Carbonyl	Glycerol	Water*	Acetal		
n-Butyraldehyde	0.537	0.854	48.8	—	0.197**	0.514+	49.1	0.340+	165	30°
"	0.549	0.662	49.8	—	0.234**	0.347+	50.1	0.315+	193	30°
"	—	—	49.3	0.762	0.348**	0.348**	49.0	0.414**	168	30°
"	Saturated with *n*-butyraldehyde.				0.88++	0.056**	50.0	0.109**	122	30°
"	Initially 4% glycerol, most of which				0.88++	0.051**	50.0	0.108**	120	30°
"	is extracted				0.75++	0.061**	50.0	0.104**	114	40°
"	—	—	52.9	0.328	0.200**	0.217**	52.7	0.110**	133	40°
"	—	—	52.8	0.339	0.210**	0.234**	52.6	0.105**	110	40°
Cyclohexanone	0.232	0.206	53.2	—	0.204+	0.177**	53.2	0.028**	41.3	30°
"	0.181	0.219	53.4	—	0.194+	0.187**	53.4	0.027**	50.0	30°

**Assuming molecular weight 18 gm.*
***By analysis.*
+Calculated from material balance.
++From solubility data.

tion is shown in Fig. 4. The values reported for glycerol, here and subsequently, are total glycerol, free and combined, to give a more accurate representation of the useful efficiency of the process. In Fig. 3 the effect of ratio on free glycerol and on acetal concentration at equilibrium has also been shown.

A typical rate curve is shown in Fig. 5. From plots of this type the values of "Ca", the rate constant, were obtained, using Equation (2). This rate constant is a linear function of acid concentration (Fig. 6) as would be expected since "C" is the concentration of hydrogen ions in the phase in which reaction occurs. This linearity is also observed when cation exchange resin is used as catalyst, but value of the proportionality constant depends on whether the increase in exchange capacity is obtained by adding more catalyst or by adding the same amount of a smaller size of resin (Fig. 7). The catalytic activity of the resin is greatly increased by reducing the average diameter of the particles, indicating that the specific surface is a factor in catalysis as well as the available acidity.

From Equation (3) a linear relation between "a" and the ratio of *n*-butyraldehyde to water would be expected. Therefore the rate constant "Ca" should be a linear function of "n" if the initial acid concentration in the aqueous phase is held constant, since hydrochloric acid remains almost entirely in the water phase. The data show a distinct curve in this relation (Fig. 8) which can probably be attributed to the inaccuracy introduced by the assumption that *n*-butyraldehyde concentration in the carbonyl phase is not affected by the formation of acetal. This applies particularly at low ratios. Not only is the carbonyl phase diluted by the formation of acetal, but analysis with Karl Fischer reagent (4) showed that the solubility of water increases, causing further dilution.

In order to determine the effect of the ratio of *n*-butyraldehyde to water on the rate constant when a cation exchange resin is used as catalyst, its effective distribution between the phases must be taken into account. A series of experiments were made with varying ratios of *n*-butyraldehyde to water and known amounts of 10-20 mesh resin as catalyst. Measured rate constants were reduced (by the use of the relation shown in Fig. 7) to the relative values they would have if: (*a*) the weight of resin per unit volume of water; (*b*) the weight of resin per unit volume of both phases; and (*c*) the weight of resin per unit volume of *n*-butyraldehyde had been kept constant as the ratio was varied. These calculated rate constants are plotted against ratio in Fig. 9. Only the calculation on the basis of the carbonyl phase gives a reasonable result. It is concluded that the resin must absorb *n*-butyraldehyde strongly and that the reaction takes place in this phase. Except at low ratios, the rate constant calculated on this basis is a linear function of the ratio of *n*-butyraldehyde to water.

A linear relation was found between log of the rate constant and the reciprocal of temperature, both with hydrochloric acid and with cation exchange resin as catalyst (Fig. 10).

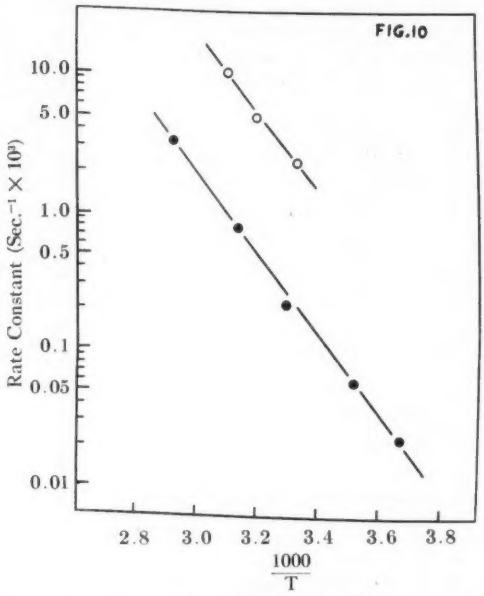


FIG. 10. *Effect of absolute temperature (T) on the rate constant.*
Conditions: ● = *ratio of n-butyraldehyde to water, 0.500; initial glycerol concentration, 4%; 0.0489 N with respect to hydrochloric acid.* ○ = *217 ml. n-butyraldehyde, 224 ml. water containing 3.90% glycerol, and 16.4 gm. of 300-325 mesh Duolite C3.*

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THE SPECIFIC HEAT OF WHEAT[1]

By P. M. Pfalzner[2]

Abstract

The specific heat of wheat at moisture contents ranging from 0% to about 15% was measured by a method of mixtures. It was found that the dependence of specific heat on moisture content can be represented by a linear law, and that the apparent specific heat of the adsorbed water does not differ appreciably from that of liquid water.

Introduction

It has been recognized for many years that any useful study of the problems arising in the storage of grain must be based on a knowledge of the physical properties of grain in bulk. Of these properties, the thermal constants are of outstanding interest since they determine the extent of any changes in temperature and moisture content of the grain during storage. The thermal conductivity of bulk grain has been found by several investigators (Babbitt (1), Bakke and Stiles (2), Kelly (3), Oxley (5)), but the specific heat has so far not been measured directly. Babbitt (1) has calculated a value for the specific heat of wheat from the values he obtained for the thermal conductivity k and the thermal diffusivity K, using the relation

$$c = \frac{k}{K\rho}$$

where ρ is the density. A direct determination of the specific heat by some calorimetric method is preferable to this procedure, while, in addition, a study of the dependence of the specific heat of wheat on the moisture content promises to give some evidence about the nature of the adsorbed water.

Apparatus

The method which was adopted for determining the specific heat is that known as "the method of mixtures" which is most commonly employed in finding the specific heats of solids. The calorimeter vessel was a Dewar flask of 500 ml. capacity. Water was used as the calorimeter fluid. The top of the flask was covered with a lid of stiff aluminum foil which had holes in it for inserting a Beckmann thermometer and a glass stirring rod. The stirrer was rotated at a constant speed by a small electric motor. Shields of aluminum foil were placed around the calorimeter to reduce the influence of room radiation.

The level of the water surface in the Dewar flask was five inches below the top edge. The stem of the Beckmann thermometer which was immersed to a

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depth of 2½ in. was thus protected for an additional length of 5 in. by the walls of the calorimeter. The thermometer was calibrated in the range 0°C. to 5°C. under the conditions of the experiment by kind arrangement with Mr. E. Green of the Metrology Section of the National Research Council. The temperature was read to 0.001°C. by viewing the scale with a telescope.

The wheat kernels were contained in a cylindrical capsule of 1 in. height, 3/8 in. radius, and 0.008 in. wall thickness. The capsule carried a screw-cover which tightened against a rubber gasket. The wheat, which weighed about 5 gm. was packed in layers between two or three perforated metal disks which served to increase the rate of heat exchange between calorimeter and capsule. In the first two series of determinations, A and B, the capsule used was of dural, while in the third, C, it was of copper.

The water equivalent of capsule and gasket was calculated from the specific heat of dural (or copper) and rubber. The specific heat of dural was extrapolated from figures for slightly higher temperatures than ours quoted in Landolt–Börnstein Tabellen, while the specific heat of the rubber gasket was calculated from the composition of the rubber as given by Mr. T. Griffith of the Rubber Laboratory of the National Research Council who kindly prepared the required rubber sheeting. The water equivalent of the calorimeter (i.e. calorimeter vessel containing 200 ml. of water, Beckmann thermometer, and stirrer) was found experimentally by measuring the rise in temperature of the calorimeter caused by immersing the capsule filled with water. The average of a large number of such determinations was taken, and the probable error in the water equivalent of the system was about 1%. This method had the advantage of eliminating any errors due to the transfer and subsequent immersion of the capsule in the calorimeter. The increase in water equivalent of the system after the addition of the capsule plus contents was allowed for in the calculations.

Method

The calorimeter was cooled to about 1°C. by introducing ice water. The capsule and wheat were placed inside a brass cylinder which was partly immersed in a large Dewar vessel containing water at room temperature. Thus the initial temperature difference between wheat and calorimeter was about 20°C.

The temperature of the capsule, i.e. of the wheat, was measured immediately prior to immersion in the calorimeter with a precision thermometer whose bulb was in contact with the brass cylinder inside which the capsule was resting. There was no danger of heat exchange between the capsule and the room in the short interval during which the capsule was transferred to the calorimeter since the room temperature and that of the capsule were nearly equal.

The moisture content of the wheat was accurately determined by the following procedure. The wheat was initially dried in a vacuum oven at 105°C. to constant weight. Thereafter the moisture content was increased by two

methods: for Series A and B the increase was brought about by exposure to humid air in a constant humidity chamber, while for C small amounts of liquid water were added to the wheat which was then placed, inside the closed capsule, into an oven at about 65°C. for 12 hr. The wheat used in all three series of determinations was a No. 1 Northern grade, A and B being from the same batch and year, while C was from a different year.

The actual procedure for obtaining measurements was as follows. The Dewar flask was filled with a mixture of water and crushed ice (200 ml., within a few tenths). The temperature was read on the Beckmann thermometer. As soon as a constant rate of temperature rise was observed, readings were taken for a period of at least six minutes at one minute intervals measured on a stop watch. This constituted the initial period. At the beginning of the next minute, the main period was begun by immersing the capsule in the calorimeter, the exact temperature of the capsule having been previously noted. The rise in temperature was again read every minute until once more a constant rate of change was attained. The readings were then continued in this final period for at least as long as in the initial period. A typical curve is shown in Fig. 1. The rate of temperature increase in the calorimeter due to room radiation was about 0.02°C. per minute; and the rise in calorimeter temperature due to the im-

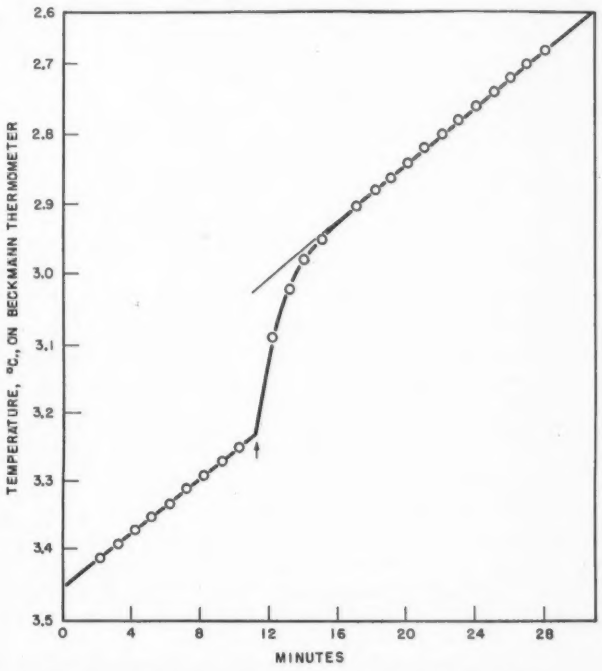

FIG. 1. *Typical curve of temperature against time for wheat at 11.1% moisture content.*

mersion of the capsule plus contents ranged from about 0.15°C. to 0.25°C. depending on the moisture content. It was found that temperature equilibrium was attained after a period of from 4 to 10 min., the longer period being due to the increased heat content of the wheat at higher moisture contents.

The average specific heat of the three lots of wheat, A, B, and C, was calculated from the relation

$$\bar{c}_{wh} = \frac{(\Delta T + \theta)W}{(t_a - t_e)m} - \frac{Wk}{m}$$

where ΔT = measured rise in temperature
θ = correction term for radiation
W = water equivalent of calorimeter
Wk = water equivalent of capsule
t_a = temperature of capsule at moment of immersion
t_e = final common temperature of capsule and calorimeter
m = mass of wheat
$\bar{c}_{wh}$ = average specific heat of wheat between the temperatures t_a and t_e.

The correction term θ is given by

$$\Delta T + \theta = t_x - t_0 + x\Delta v + \frac{\Delta n - \Delta v}{t_n - t_v}\left(\sum_{1}^{x-1} t + \frac{t_0 + t_x}{2} - xt_v\right)$$

where t_x, t_0 = last and first temperature respectively of main period
x = number of time intervals in main period
Δv, Δn = mean temperature drop between two readings of the initial and final period respectively
t_v, t_n = mean temperature of the initial and final period respectively
$\sum_{1}^{x-1} t$ = sum of temperatures during the main period with the exception of t_0 and t_x.

The following constants were used in calculating the water equivalent of the calorimeter and the specific heat of the wheat: average specific heats of water, dural, copper, and rubber, between 2°C. and 20°C.: 1.002, 0.217, 0.0913, 0.35 respectively, and average specific heat of water between 2°C. and 3°C.: 1.005.

Results and Discussion

The accuracy of specific heat measurements by the method of mixtures is largely dependent on the accuracy obtainable in determining the temperature rise of the calorimeter. Hence it is desirable that this temperature rise should be as large as is practicable; this will be the case if (*i*) the initial temperature difference between calorimeter and capsule is large, and (*ii*) the heat capacity of the calorimeter is small relative to that of the capsule and contents.

It is usual to raise the temperature of the body whose specific heat is to be measured above that of the calorimeter by heating it in some manner. How-

ever, in the particular case of wheat containing moisture, the following effects must be considered. When the warm capsule is suddenly immersed in the cold calorimeter, there are, in addition to the heat content of the capsule, wheat, and bound water, three further sources of energy which may contribute to the observed change in temperature of the calorimeter:

(*a*) the temperature gradient between the center of the capsule and the walls during the cooling of the wheat may cause migration of moisture in the wheat from warm to cold regions,

(*b*) the moisture of the air trapped in the capsule may condense on the cool walls,

(*c*) cooling the whole mass of wheat may increase the degree of binding of water molecules on the wheat.

With regard to (*b*) a simple calculation may be made to show that the effect will be negligible. At 75% relative humidity and 21°C. the absolute humidity of the air is about 12.5 gm. per cu. m.; the volume of the capsule is about 7.2 cc., and hence, assuming that as much as half that volume is air space, there will be about 5×10^{-5} gm. of water vapor in the air contained in the capsule. If all of this moisture were to condense and cool to 0°C. the total energy given up would be about 0.02 calories. The amount of water sorbed in about 5 gm. of wheat in equilibrium with air at 21°C., 75% R.H. will be about 0.6 gm., and when this wheat is cooled to 0°C., the wheat will release about 30 calories (specific heat about 0.3) and the bound water will release between 6 and 12 calories (specific heat may be taken as low as 0.5 and as high as 1.0), i.e. a total of at least 36 calories. Thus the effect of moisture from the air condensing out when cooling through 20°C. will certainly be less than 0.06% of the total energy released, and this is well within the experimental errors.

The effect (*a*) is less definite and will be discussed in a qualitative manner. Supposing again that the temperature difference between capsule and calorimeter is at most 20°C., then a maximum gradient of 20°C. will exist initially between the center and outside of the capsule, i.e. over a distance of 3/8 in. It is found, however, that this gradient decreases to about half its maximum value in the first minute after immersion, and after at most 10 min. complete temperature equilibrium is attained. In experiments on the thermal conductivity of wheat, Moote (4) found that when a gradient of about 20°C. was maintained over a depth of five inches of wheat, the moisture content on the warm side dropped to about half its initial value after a period of more than 300 hr., while the moisture content on the cool side rose by about 10%. Now in our case the initial gradient is much steeper but it is rapidly diminishing and drops to zero within 10 min. Thus it is reasonable to assume that very little moisture migrates within the wheat from place to place, and that the resultant energy change will be quite negligible for the dimensions of capsule and temperature gradient actually employed in the experiments.

(*c*). This effect will involve a true energy change of the wheat and bound water and should thus be considered part of the real energy content. The

occurrence of this effect is based on the assumption that the binding energy of the water molecules differs in different layers or regions of the adsorbed film. When the wheat is cooled some of the sorbed molecules are bound more tightly, i.e. give up part of their energy. The assumptions about the nature of the binding forces are outside the scope of this paper.

In order to obtain a large temperature rise in the calorimeter, one can also, besides heating the body to be immersed, resort to using a large mass. The objection to this is, of course, that a large mass will increase the time required to establish temperature equilibrium between wheat and calorimeter, and this will increase the necessary correction for room radiation which is only approximate. A large mass will also introduce complications due to the effect discussed under (*a*). All these considerations together led to the choice of actual temperature gradient, mass of wheat, and of water in the calorimeter. It was also felt that by cooling the calorimeter and keeping the wheat at room temperature, its moisture content would be more accurately known prior to immersion.

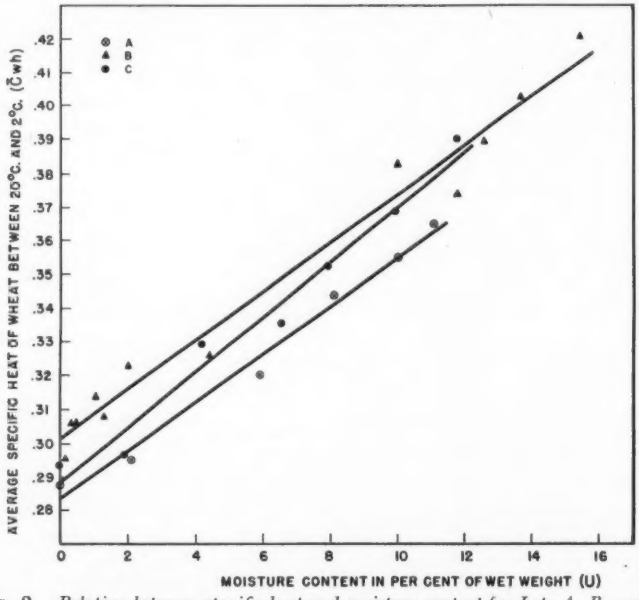

FIG. 2. *Relation between specific heat and moisture content for Lots A, B, and C.*

In Fig. 2 the specific heat values found for the lots A, B, and C are plotted against the moisture content in per cent of wet weight. Assuming that the specific heat of wheat plus bound water is the sum of the specific heats of each of the components in the proportion of their masses, the relation between specific heat and moisture content can be expressed by the linear equation,

$$\bar{c}_{wh} = \frac{U}{100}\bar{c}_w + \frac{100 - U}{100}\bar{c}_0$$

where $\bar{c}_w$ is the average specific heat of water and $\bar{c}_0$ is the average specific heat of dry wheat. This is equivalent to

$$\bar{c}_{wh} = \bar{c}_0 + NU$$

where N is some constant and is itself given by

$$N = (\bar{c}_w - \bar{c}_0)/100.$$

This approach implies, of course, that the specific heat of the sorbed water is constant for all moisture contents.

The equations for the best straight lines through the experimental points were obtained by the method of least squares and they are

$$\begin{aligned}
\bar{c}_{wh} &= .283 \pm .001 + (.00724 \pm .00015)\, U \\
\bar{c}_{wh} &= .301 \pm .001 + (.00733 \pm .00012)\, U \\
\bar{c}_{wh} &= .288 \pm .002 + (.00828 \pm .00022)\, U
\end{aligned}$$

for the lots of wheat A, B, and C respectively. Babbitt (1) has calculated a value for $\bar{c}_0 = 0.31$ from his measurements of the thermal conductivity and diffusivity, and this is seen to be in good agreement with our values which range from 0.283 to 0.301. It will be noted that the $\bar{c}_0$ values for Lots A and B which were from the same batch of wheat differ by almost 10% while the slopes differ only by slightly more than 1%. Wheat C which was taken from a different batch has an intermediate value of $\bar{c}_0$ but has a considerably steeper slope.

Babbitt assumed in his calculations that the specific heat of bound water is equal to that of free water. We can readily obtain a value for the specific heat of the bound water, for from

$$N = (\bar{c}_w - \bar{c}_0)/100$$

we find that

$$\begin{aligned}
\bar{c}_w &= 1.007 \pm .036 \\
&\quad\ 1.034 \pm .032 \\
&\quad\ 1.116 \pm .052
\end{aligned}$$

for A, B, and C respectively. It is seen that the specific heat of the sorbed water is slightly higher than that of free water in all cases, but only for C is the difference apparently significant. Whether this may be due to a real difference or to the fact that the wheat in C was wetted with liquid water and then heated while contained in the closed capsule, instead of being conditioned in humid air as was the case for A and B, it is not possible to decide from our data.

The results here obtained are in fairly good agreement with measurements on the specific heat of water adsorbed on cellulose as reported by Shipley, Campbell, and Maass (6). Their values for the specific heat of sorbed water measured between +5°C. and +25°C. range from almost 1.13 at 2% moisture content to 1.11 at 12% with a minimum of about 1.08 at 5%. They suggest that the specific heat as found in their experiments is composed of two separate

contributions, one from the heat content of the sorbed water itself and the other from the change in the binding energy between the water molecules and the adsorbent.

It may thus be maintained that the true specific heat of sorbed water is less than unity which is what one expects since sorbed water molecules will be under constraint, i.e. will have fewer degrees of freedom than free water, and that there appears an additional contribution from the energy of binding. In the present experiments, then, the sum of these two contributions would lie between unity and 1.12.

Conclusion

The specific heat of wheat is, to a good approximation, a linear function of the moisture content expressed in per cent of the wet weight. The apparent specific heat of bound water does not differ appreciably from that of free water over the range of moisture contents and temperatures investigated.

Acknowledgment

My sincere thanks are due to Dr. J. D. Babbitt for his interest and constructive criticism, and to Mr. Roderick Ross who performed some preliminary experiments.

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